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Physiologic Specialization and Cytology of Rhizoctonia Solani From Cotton.

Mohamed-nagy Mahmoud Shatla
Louisiana State University and Agricultural & Mechanical College

PHYSIOLOGIC SPECIALIZATION AND CYTOLOGY OF *RHIZOCTONIA SOLANI* FROM COTTON

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Mohamed-Nagy Mahmoud Shatla
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ABSTRACT

Fifty-two varieties, strains, lines, and species of cotton have been tested to determine their reaction of 3 isolates of Rhizoctonia solani Kühn from cotton. Their reaction varied from resistant to susceptible. Resistance of cotton plants to R. solani infection behaved as any other biological phenomenon showing a normal curve. Temperature was one of the determining factors for behavior. Twelve differential varieties of cotton were chosen to establish physiologic specialization of 3 isolates. It was suggested, however, that a larger number of varieties should be tested against a larger number of isolates to well establish this phenomenon.

Cytology of R. solani hyphae was studied by the light, phase, and electron microscopy. Stained preparations of somatic hyphae showed that the average width of hyphae was 7 u and that mycelial cells were multinucleate. The number of nuclei in hyphal tip cells, sometimes reaching 15 was greater than cells behind the tip cell. Average size of a nucleus in the resting stage was 1.6-1.7 u. In old cells, back from the tip cell, nuclei were lesser in number and restricted to hyphal cell walls. Nuclear degeneration was observed in old cells. The mode of division in somatic nuclei was mitotic. Even though mitotic figures were observed, no spindle apparatus was found. The (n) chromosome number appeared to be 4. Observations under the phase microscope confirmed that of stained preparations.

The sequence of events in division could not be observed in living material, however, a dividing nucleus was found. A nuclear membrane was detected.

The ultrastructure of R. solani hyphae was revealed in electron microscope studies. The ultrastructure of the nuclear envelope and presence of oil vacuoles was recorded for the first time. Structure of the cell wall, mitochondria, and septal pore apparatus was confirmed.

INTRODUCTION

Rhizoctonia solani Kühn is one of the most important pathogens in the cotton seedling disease complex (58). Estimated losses due to seedling disease in Louisiana reached 5 per cent of the crop in 1962 and 1963 (46, 47). For 3 successive years in Louisiana R. solani was the pathogen most frequently isolated from diseased cotton seedlings collected at random in cotton growing areas of the state. It accounted for 1/2-2/3 of the pathogens involved (72). R. solani consists of a heterogenous group of races or strains differing in growth characters and pathogenicity (34). Attempts to establish physiologic specialization of R. solani isolates on cotton have been largely neglected. The few reports that have been made were either misleading or failed to meet the criteria of physiologic specialization. Differentiation of races of R. solani according to their pathological effects on cotton varieties, has not been reported. It is the main object of the first part of this manuscript to establish physiologic specialization of R. solani on cotton. It was believed that such a study would be of great significance as a step forward in differentiating R. solani races. This may help, also, in breeding for resistance and in epidemiology studies.

The second part of this manuscript deals with the cytology and mode of nuclear division in somatic hyphae of R. solani.

Hundreds of publications deal with the mode of division of sexual

cells in fungi. These studies confirm and report occurrence of meiotic and mitotic nuclear division in such cells. The mode of nuclear division in somatic cells of fungi has received little attention until recently. There have been contradictions among investigators about the mode of division of these somatic nuclei. Some supported the idea of the occurrence of mitotic nuclear division similar to that reported for higher plants. Others believed in direct division.

It was the objective of the second part of this study to determine mode of division in somatic cells of R. solani and nature of the ultrastructure of somatic hyphae. The work reported here may be of importance to people studying the origin and evolution of fungi.

LITERATURE REVIEW

Part I

Physiologic Specialization and its Significance

It has long been known that within species of many fungi there were lines or races morphologically indistinguishable but physiologically different (14).

The discovery by Eriksson (59) of specialized races of rusts was one of the important developments in plant pathology. His demonstration in morphological species of Puccinia graminis Pers.; P. glumarum (Schmidt) Erikss. and Henn.; P. dispersa Erikss. and Henn.; and P. coronata Ida. of subdivisions which could be distinguished from one another by their ability to grow on some hosts and not on others, stimulated many workers to investigate the extent and significance of host specialization among various other groups of parasitic fungi.

Investigations of physiologic specialization in plant pathogens, especially those which attack cereals, vegetable crops and other economic plant groups, continued to occupy a foremost place in plant pathology. Many workers in various parts of the world have contributed to our knowledge of host specialization and have emphasized the importance of recognition of this phenomenon in breeding for disease resistance in crop plants (60).

The primary basis for evidence of physiologic specialization was the behavior of different cultures of parasitic fungi on particular hosts. Recognized hosts may be infected by some collections of a morphological species of a fungus and not by others. Obligate parasitic fungi, such as rusts and powdery mildews, must be cultured on living plants. One of the problems with these fungi was the reaction of suitable hosts for differentiation of specialized races. If such exist, races may be distinguished by the type and degree of infection.

Many parasitic fungi, such as smuts, and various species and genera of the Ascomycetes and Fungi Imperfecti, however, may be cultured in the laboratory media, and it was possible to distinguish strains or races by differences in their growth characteristics.

Such strains were called "cultural races" and distinguished from "pathogenic races," which were differentiated by their capacity to infect living plants.

In culture, it was possible to observe the influence of various substances on rate and extent of growth. Character of growth, its consistency, whether uniform or sectoring, topography of the surface, color, etc., were all characters used for distinguishing between different strains. Economically the most important of these characters was pathogenicity (59).

Stakman (80) mentioned that these races have been called many names, good and bad, but when the most conspicuous or important

distinguishing characters were in behavior, races usually were designated as physiologic forms, biologic forms, physiologic races, specialized races, parasitic strains, or some other kind of strains.

The term "physiologic race" seemed most appropriate to specify the particular type of behavior that differentiated the races. The more precisely descriptive terms "parasitic race," "cultural race," etc., however, may be more useful. Physiologic races did not always differ from each other in physiologic characters only, but many also differed in morphology.

Much has been done to elucidate the nature of physiologic races. In some cases a race may consist of a single biotype, while in others it comprises many biotypes.

There was strong evidence that different collections identified as the same physiologic form usually belong to the same biotype, although there was always the possibility that two collections that appeared identical on differential varieties might prove different on certain other varieties or in characters other than pathogenicity. In general, however, physiologic races of rusts probably comprise single biotypes. Just what a physiologic race comprised in many Fungi Imperfect and certain other fungi with multinucleate spores and hyphal cells can be determined only if the complete nuclear history is known.

Churchward (15) reported that physiologic races may arise by mutation or hybridization in nature or be introduced from other areas.

Physiologic specialization was, therefore, important in a consideration of establishment of plant quarantines, in epidimiology studies,

and in production of disease resistant varieties. The mode of inheritance of resistance within hybrids may change with the introduction or occurrence of new races. Hence, it is essential that plant breeders should know what races are present, their distribution, pathogenic capabilities and possibility for the origin and introduction of new races. Churchward (15) further mentioned that it was not surprising to find pathogenicity tests were the chief means of differentiating races, since breeders and growers were concerned mostly with effects of races of the pathogen upon the crop.

The matter of breeding disease-resistant varieties was made more difficult by the fact that the reaction of certain varieties to a given physiologic race may differ with varying environmental conditions.

Smith (77) has pointed out that the wheat variety, Hope, when spring sown in Washington was resistant to several races of bunt, but became moderately susceptible when fall sown.

Waterhouse (59) made the significant discovery that the specific reaction of a host to certain physiologic forms of Puccinia graminis tritici varied under winter and summer conditions. He found, in some cases, that a given variety was susceptible to certain specialized forms in hot, summer months and resistant in winter months. Waterhouse (59), found similar variations in the behavior of certain forms of Puccinia graminis avenae, P. tritiçina and P. simplex. With the latter species, 14 susceptible varieties of barley gave the same results during both summer and winter. Eight varieties, however, resistant under winter conditions were susceptible during the summer period.

Petursen (54) found that red rust-proof oats was resistant to form 4 of Puccinia coronata avenae at 14°C and susceptible at both 21°C and 25°C.

Physiologic Specialization in R. solani

It has been shown that R. solani is comprised of a heterogenous group of races or strains differing in growth characters and pathogenicity (34). R. solani isolates have been shown to differ in their pathogenicity, host range, morphological characters, utilization of chemicals, secretion of enzymes, optimum temperature requirement, optimum pH, and tolerance to chemicals (9, 40, 44, 45, 53, 66, 73, 74, 82, 84, 85). Some of these reports had titles implying reports on physiologic specialization of R. solani, then dealt with differences in morphological characters, utilization of chemicals, etc. (45).

The attempt to establish physiologic races of R. solani on the basis of pathological reaction on differential host plants has been largely neglected. LeClerg (39) tried to differentiate races of R. solani on the bases of parasitism on several crop plants. He studied 29 isolates from sugar beets and potatoes. There was so much variation among the results of repeated tests that races could not be accurately distinguished by this method. Attempts were made to identify races by direct inoculation of 6 varieties of beans, again, the degree of variability was too great to warrant using this method for identification of races.

Walker (89) reported on the pathogenicity towards cotton seedlings

of two strains of R. solani from cotton, and one each from cabbage and potato. The cabbage strain had an optimum temperature of 24°C and was found to be practically innocuous to cotton, only one plant out of 102 being killed, whereas the potato strain was almost as virulent as cotton strains. The optimum temperature for the potato strain was 27°C.

Hunter, Staffeldt, and Maier (35), using 3 isolates of R. solani which were known as mildly, moderately, or highly pathogenic, found that their pathogenicity level and virulence on Acala cotton seedlings varied according to temperature. The optimum temperature for infection by the mildly pathogenic isolates was 24°C, that for the moderately pathogenic isolate was 32°C, while 24°C, 27.5°C and 32°C had no effect on the virulence of the highly pathogenic isolate.

Teng (83) showed that 50 cotton varieties tested for their reaction to Corticium solani, one of Gossypium hirsutum, two of G. arboreum, and 47 of G. nanking proved equally susceptible to the fungus. Fahmy (27) reported that cotton soreshin disease was present wherever cotton was grown in Egypt, and was most severe in heavy, moist soils. All varieties of cotton were susceptible, if sown early in the spring when temperatures were unfavorable for the rapid development of seedlings.

Fulton, Waddle, and Bollenbacher (30) screened 650 strains and varieties of cotton for cold tolerance and resistance to R. solani, Pythium ultimum, Colletotrichum gossypium, and Thielaviopsis basicola. All lines were screened for cold tolerance at 60°F before being tested for disease

resistance. They found fairly good tolerance to all fungi tested, except R. solani. No cotton line, however, was found highly resistant to all 4 fungi used. Some showed good tolerance to all but R. solani. Fulton, et al. (30) concluded that in most Gossypium spp. germ plasm had no outstanding resistance to the seedling disease fungi used in this screening test and that there was no consistent correlation between cold tolerance and disease resistance. They further mentioned that the most promising source of resistance to P. ultimum, C. gossypii, and T. basicola was found in G. thurberi, G. arboreum and a G. barbadense x G. hirsutum hybrid. The only indication of promising resistance to R. solani was in some cold tolerant lines originating in Yugoslavia.

Maier (42) separated 10 strains of R. solani from 245 isolates on the basis of their cultural variation and ability to parasitize cotton. Limited cultural variations were found among the 10 strains which also varied in pathogenicity from nonpathogenic to highly pathogenic. These strains maintained their respective levels of pathogenicity through several pathogenicity trials on both Upland and American-Egyptian cottons.

Maier and Staffeldt (43) found that 12 R. solani isolates differed in cultural characteristics in laboratory studies. Their pathogenicity on Acala cotton ranged from nonpathogenic to highly pathogenic.

Shatla and Sinclair (73) reported that 36 R. solani isolates varied in their tolerance to pentachloronitrobenzene (PCNB) from highly tolerant to sensitive and that pathogenicity was correlated with tolerance.

From these reports it appeared that there was a lack of information concerned with the identification of physiologic races of R. solani according to their pathological effect on different cotton varieties.

The objectives of this part of this manuscript were:

1) To evaluate cotton varieties, lines, strains, and species for their resistance and susceptibility to 3 isolates of R. solani.
2) To determine the effect of temperature on the reaction of these cotton lines to R. solani.
3) To determine if irradiation of cotton seeds had some effect on the tolerance level of these cotton lines to R. solani.
4) To establish a set of differential varieties of cotton for the identification of R. solani isolates and set a criterion for their pathological evaluation.

MATERIALS AND METHODS

Part I

Resistance to Rhizoctonia solani Kühn of varieties and strains of cotton belonging to major cultivated and wild species was determined to establish physiologic specialization in the fungus. The 3 isolates of R. solani used were: T, 6, and OP-2, isolated by J. B. Sinclair in the years 1959, 1960 and 1961, respectively, and maintained in pure culture since that time.

Cotton varieties, strains and species used in this study are listed in Table 1.

The Upland cotton varieties (Gossypium hirsutum) were obtained from Jack Jones of the Department of Agronomy, Louisiana Agricultural Experiment Station. Varieties of Gossypium barbadense were obtained from C. V. Feaster, U.S.D.A. Agricultural Research Service, Cotton Research Center, Tempe, Arizona. Yugoslavian strains were obtained from B. A. Waddle, Department of Agronomy, University of Arkansas, Fayetteville. Six wild species were obtained from Meta Brown, Texas A. & M. College, College Station.

Several lines of cotton from irradiated seed were obtained from M. Constantin, University of Tennessee (Table 2). Several lines of glandless cotton seed were obtained from S. C. McMichael, U.S.D.A. Agricultural Research Service, Cotton Research Station, Shafter, California.

Table 1. List of cotton species and their varieties and strains tested for their resistance to 3 strains of *Rhizoctonia solani*.

No.	Species	Variety or Strain
1	*Gossypium barbadense*	Amsak
2	" "	Old Pima
3	" "	Pima S-1
4	" "	Pima S-2
5	" "	Prog. S x P
6	" "	Menoufi
7	*Gossypium hirsutum*	Empire WR-61
8	" "	Fox 4
9	" "	Dekalb 108
10	" "	Carolina Queen
11	" "	Stoneville 7-A
12	" "	Coker 100 A
13	" "	Rex SLL
14	" "	Dekalb 220
15	" "	Auburn 56
16	" "	DPL 15
17	" "	Stardel
18	" "	Wescot
19	" "	Dixie King
20	" "	DPL Smooth Leaf
21	" "	CB 30 51 Lot 1
22	" "	CB 30 51 Lot 2
23	" "	CB 30 35
24	" "	CB 30 34
25	" "	Early Upland
26	*G. anomalum*	
27	*G. davidsonii*	
28	*G. gossypioides*	
29	*G. raimondii*	
30	*G. stocksii*	
31	*G. thurberi*	
32	*G. hirsutum* (?)	Glandless 1
33	" "	Glandless 16-25
34	" "	Glandless Y257

Table 2. List of interspecific hybrids from irradiated cotton seed (G. hirsutum x G. barbadense) tested for resistance to 3 strains of R. solani. The number, row designation, parents and irradiation treatment are given.

No.	Row No.	Parents[1]	Irradiation treatment in r /hr post pollination
1	9	M8 x Coastland	None (check)
2	29	" "	"
3	257	" "	"
4	317	" "	"
5	345	" "	"
6	7	" "	400/216
7	252	" "	500/98
8	220	" "	500/100
9	223	" "	500/100
10	300	" "	500/145
11	364	" "	500/146
12	319	" "	800/97
13	191	" "	800/98
14	226	" "	1000/98
15	19	" "	1600/216
16	22	" "	1600/216
17	24	" "	1600/216
18	295	M11 x Coastland	500/96
19	272	" "	500/96
20	279	" "	500/146
21	200	" "	800/98

[1] M8 was strain of DPL-14 developed by doubling the chromosome number of a haploid plant (G. hirsutum).
M11 was a strain of Empire developed as M8 (G. hirsutum).
Coastland was a strain of Sea Island variety (G. barbadense).

The Upland, Sea Island, and the Yugoslavian varieties were tested: (1) under greenhouse conditions; (2) under controlled temperatures of 20-24°C; and (3) under field conditions at Northeast Louisiana and Red River Valley Branch Experiment Stations. Wild species and irradiated seed were tested under greenhouse conditions; three lines of glandless cotton seed were tested under field conditions.

Several procedures were used to determine the reaction of these cotton varieties and species to R. solani isolates according to the space and amount of seed available for testing.

Preparations and procedures for testing were the same in every approach used in this study. R. solani isolates were grown in potato-sucrose broth (PSB) for 10-15 days. PSB was prepared by using the extract from 200 g of potatoes, plus 20 g of sucrose in 1 liter of distilled water. Cultures were incubated at room temperature (22-32°C) in a closed cabinet. After this period broth was decanted off to eliminate the influence staling products might have on experimental results. The fungus mat was broken into pieces and placed in a Waring blender with 200 cc distilled water and mixed for about 30 sec. The resulting suspension was used as a source of inoculum.

The evaluation of the Upland, Sea Island and Yugoslavian varieties for their resistance and susceptibility to R. solani was carried out in an air-conditioned room at 20-24°C. Ten seeds of each variety were planted in a 4-inch clay pot filled with steam-sterilized soil. Each pot was

considered a replicate and each variety was replicated 4 times. For each variety, 4 pots without inoculation were used as checks. At the time of planting about 5 cc of mycelial suspension was added to each pot. After about 20 days, and after the first true leaf appeared the number of healthy plants that survived per pot were counted and the mean obtained. The mean percentage of surviving healthy plants was used as a criterion to express resistance or susceptibility of these cotton varieties to R. solani isolates.

Under greenhouse conditions, Upland and Sea Island varieties were evaluated by planting 50 seeds of each variety in each of 5-7 rows in greenhouse flats of sterile soil (only 25 irradiated seeds were planted in each of 4 rows because of the shortage in the seed number). Each row in a flat was considered a replicate. Five replicates were used, only 4 replicates were used in the irradiated seed. Varieties were distributed into a randomized block design.

At the time of planting about 15-20 cc of the mycelial suspension was added to each flat, then flooded with water to assure the even distribution of the inoculum. Noninoculated flats served as checks.

After the first true leaf appeared (between 17-22 days), the number of healthy seedlings was counted and the percentage of healthy surviving plants for each variety was calculated.

Yugoslavian lines and wild species were tested under greenhouse conditions using the same procedure as described for laboratory testing. Seed of wild species was treated by concentrated sulfuric acid before planting to facilitate and enhance the germination.

These varieties and lines, in addition to 3 lines of glandless cotton seeds (except the wild species and the irradiated lines), were tested under field conditions by planting 200 seeds for each variety. After 20-25 days, stand counts of surviving healthy plants were counted and the percentage of survival was determined based on percentage of germination for each variety.

RESULTS

Part I

Results from testing various varieties, lines and species of cotton for resistance to 3 isolates of R. solani showed that 14 varieties of Upland cotton tested first under greenhouse conditions showed a great variation in reaction (Fig. 1). Isolate OP-2 appeared the least pathogenic of the 3 isolates on all cotton lines tested except for the wild species. Variety Wescot showed the highest resistance to this isolate with a reading of 90 per cent survival. Deltapine 15 and Coker 100A appeared to be the most susceptible Upland varieties tested. The percentages of healthy plants for these varieties were 18.7 and 19.0, respectively. Other varieties such as Dixie King and Deltapine smooth leaf had 80.0 per cent survival, and were considered as resistant. Carolina Queen, Stoneville 7-A and Auburn 56 appeared to be moderately resistant.

The reaction of these Upland varieties to this isolate of R. solani under controlled temperature (20-24^{o}C) likewise showed a great variation in respect to resistance and susceptibility to this isolate. Under these conditions the variety Wescot, which showed the highest resistance under greenhouse conditions (temperature 28-33^{o}C), was the least resistant variety. The survival of healthy plants reached 6 per cent while Deltapine 15 was about 8 per cent. The most highly resistant of these varieties under

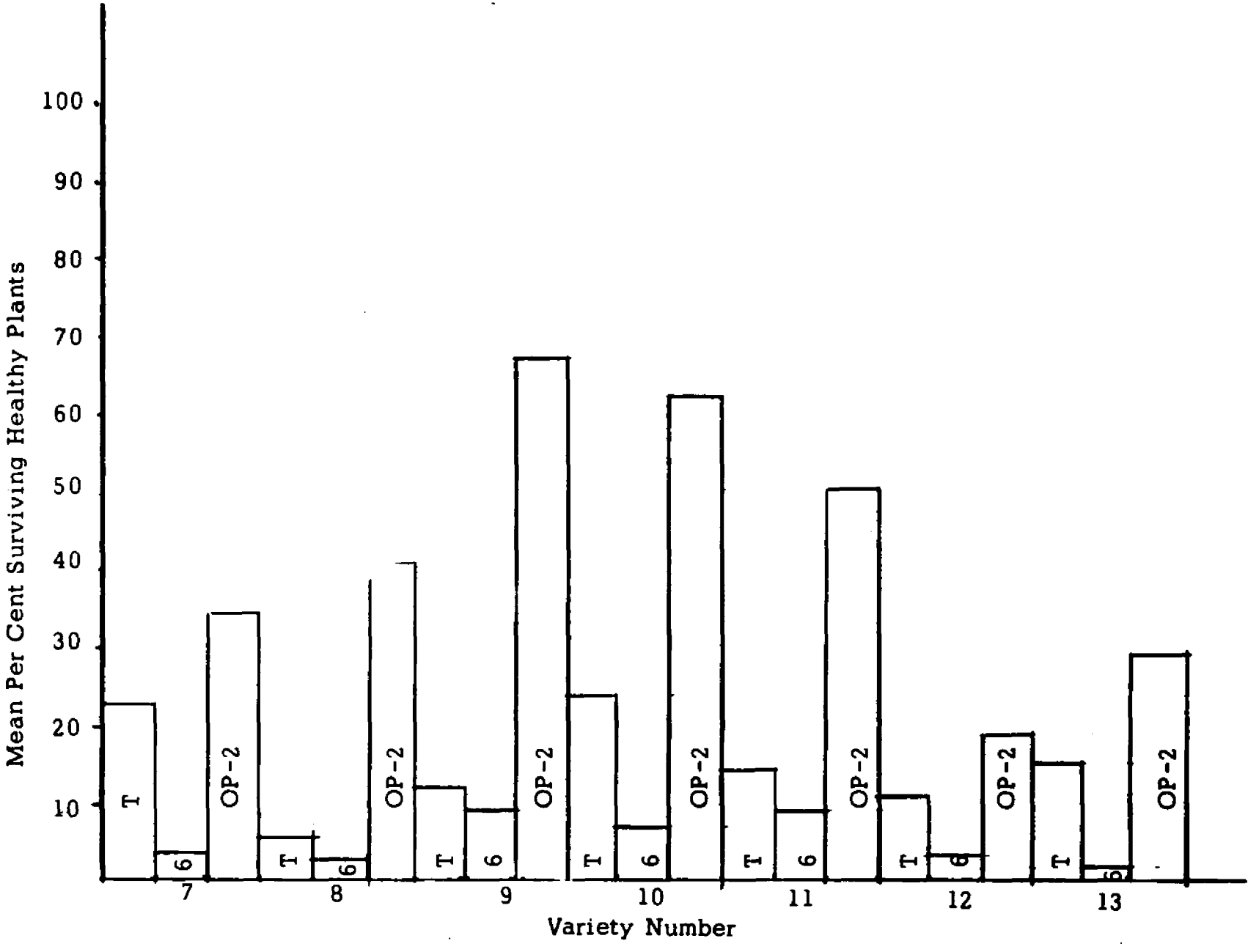

Figure 1. Mean (5 replicates) per cent of surviving healthy plants for Upland cotton (G. hirsutum) varieties indicated by number grown under greenhouse conditions in soil infested with either isolate 6, T, or OP-2 of R. solani.

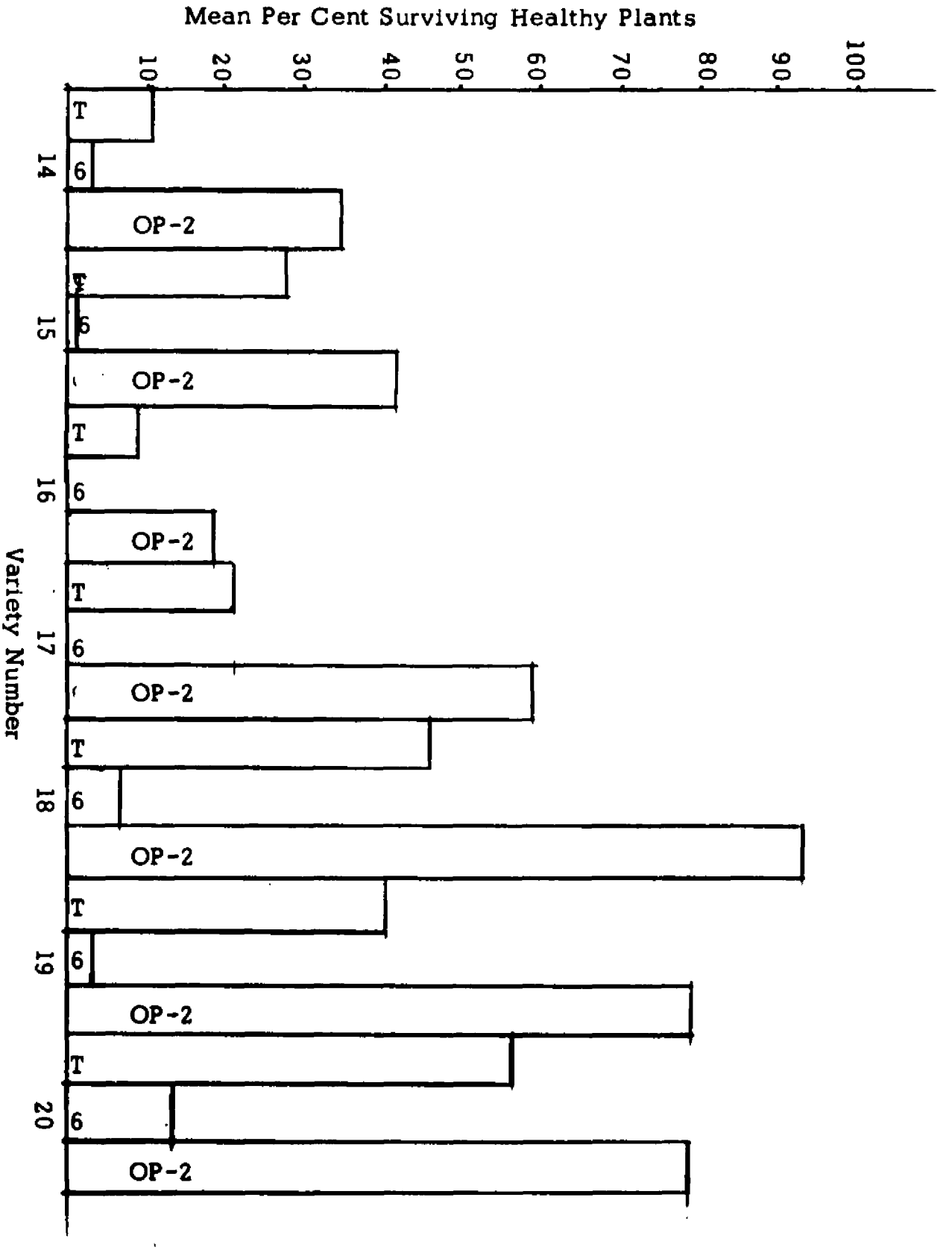

Figure 1. Continued.

controlled temperatures of 20-24°C were Coker 100A and Dekalb 220 which appeared as susceptible under greenhouse conditions. The survival of healthy plants in the infested pots reached 78 per cent. Under these conditions Deltapine smooth leaf appeared to be susceptible. It had 18 per cent survival.

The reaction of Upland varieties to 3 isolates of R. solani under controlled temperatures is given (Fig. 2).

The reaction of Upland varieties to isolate T under greenhouse conditions showed great variation. Deltapine smooth leaf, Wescot, and Dixie King were obviously tolerant to this isolate. The survival of healthy plants for these varieties reached 60 per cent, while for varieties such as Fox 4, Dekalb 220, Deltapine 15, their percentage did not exceed 10 per cent. The other varieties tested fell between these 2 extremes. Under controlled temperatures in the laboratory, it was noticed that Wescot, Stoneville 7-A, Coker 100A, and Auburn 56 had the greatest tolerance to this isolate, while varieties Rex SLL, Carolina Queen, Stardel, Dekalb 108, DPL smooth leaf were very susceptible. Other varieties tested fell in between these 2 extremes.

The behavior of these Upland varieties to isolate 6 under greenhouse conditions showed that all varieties tested were highly susceptible to it. There was, however, a difference in degree of susceptibility. Deltapine smooth leaf had 13 per cent survival, while varieties such as Stardel and Deltapine 15 had no plants which survived. Under controlled temperatures

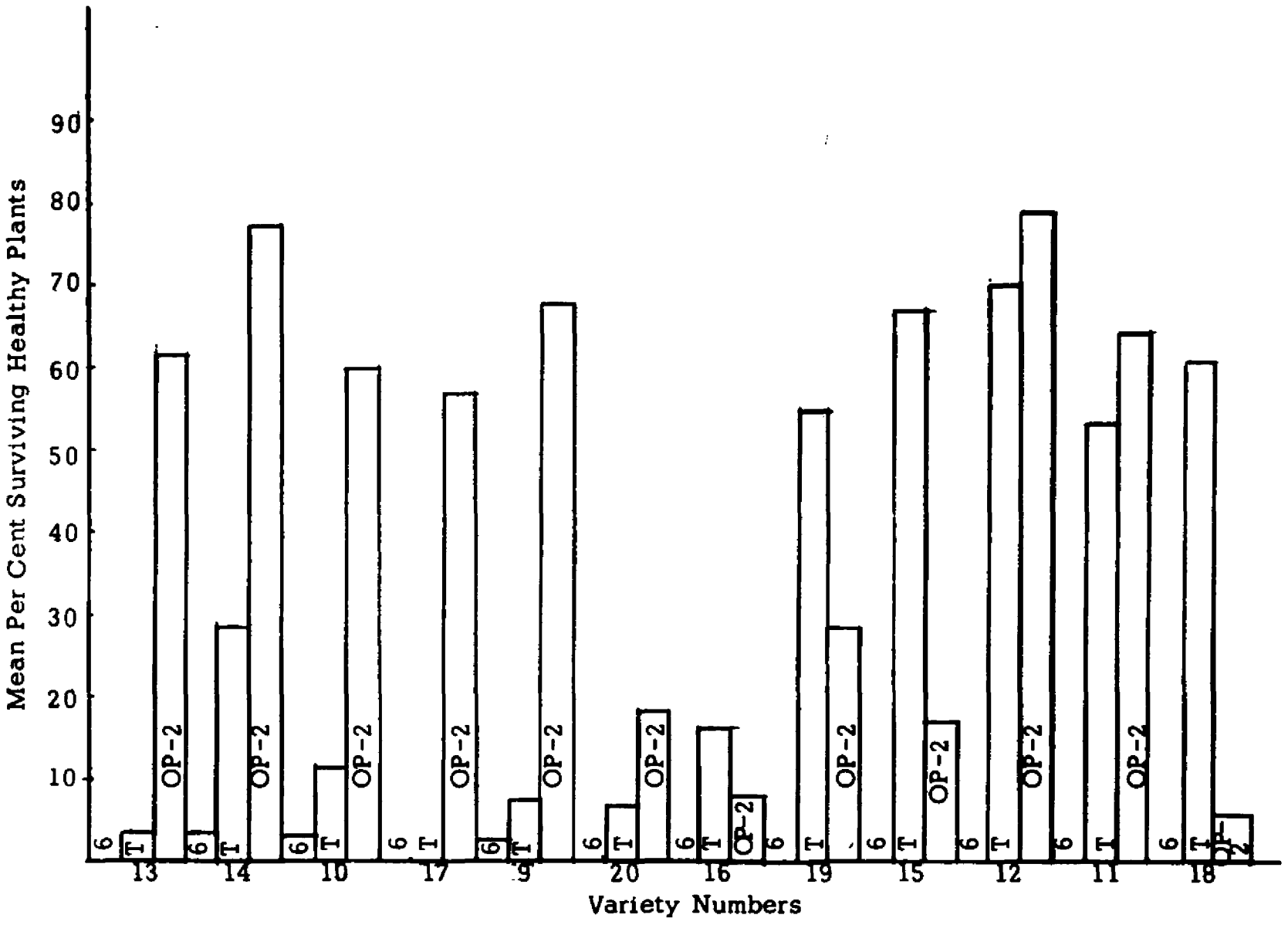

Figure 2. Mean (4 replicates) per cent of surviving healthy plants for Upland cotton (G. hirsutum) varieties indicated by number grown under controlled temperature (20-24°C) in soil infested with either isolate 6, T, or OP-2 of R. solani.

these Upland varieties showed 3 per cent survival. No noticeable variations were found under these conditions.

The reaction of Sea Island cotton varieties to the 3 isolates of R. solani under greenhouse conditions showed that all varieties had a high tolerance to isolate OP-2 (Fig. 3 and 4). Under controlled temperatures of the laboratory, considerable variation in the reaction of this isolate among the varieties was recorded. Pima S-1 had the lowest survival with only 14 per cent, while Old Pima had the highest with a reading of 84 per cent. The other varieties fell between these two extremes.

The reaction of the Sea Island varieties to isolate T showed a great variation under greenhouse conditions. Prog. S x P showed the highest tolerance with an index of about 50 per cent, while Menoufi reached 14 per cent. The other varieties fell between these 2 extremes. Under controlled temperatures Menoufi had the highest survival of 28 per cent, while Pima S-2 and Prog. S x P had 0 per cent, and the rest of the varieties fell in between these 2 extremes.

Sea Island varieties were most susceptible to isolate 6 both under greenhouse conditions and controlled temperature in the laboratory. The percentage of healthy plants that survived under greenhouse conditions varied from 1-9 per cent, while under the controlled temperature conditions no single plant survived.

From these results it appeared that the Sea Island cotton varieties, as a group, were more tolerant to R. solani than the Upland group. Under

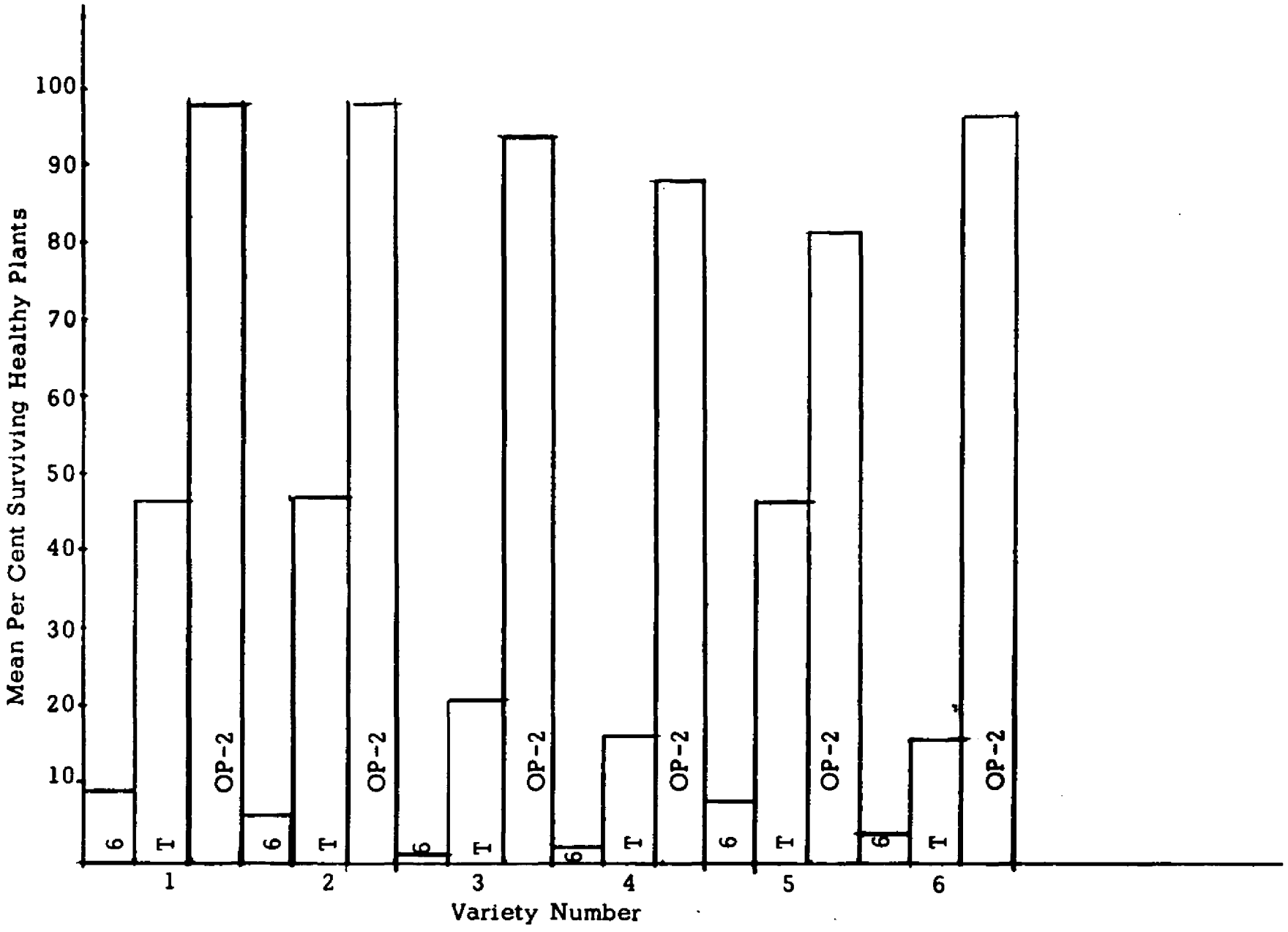

Figure 3. Mean (5 replicates) per cent of surviving healthy plants for Sea Island cotton (G. barbadense) varieties indicated by number grown under greenhouse conditions in soil infested with either isolate 6, T, or OP-2 of R. solani.

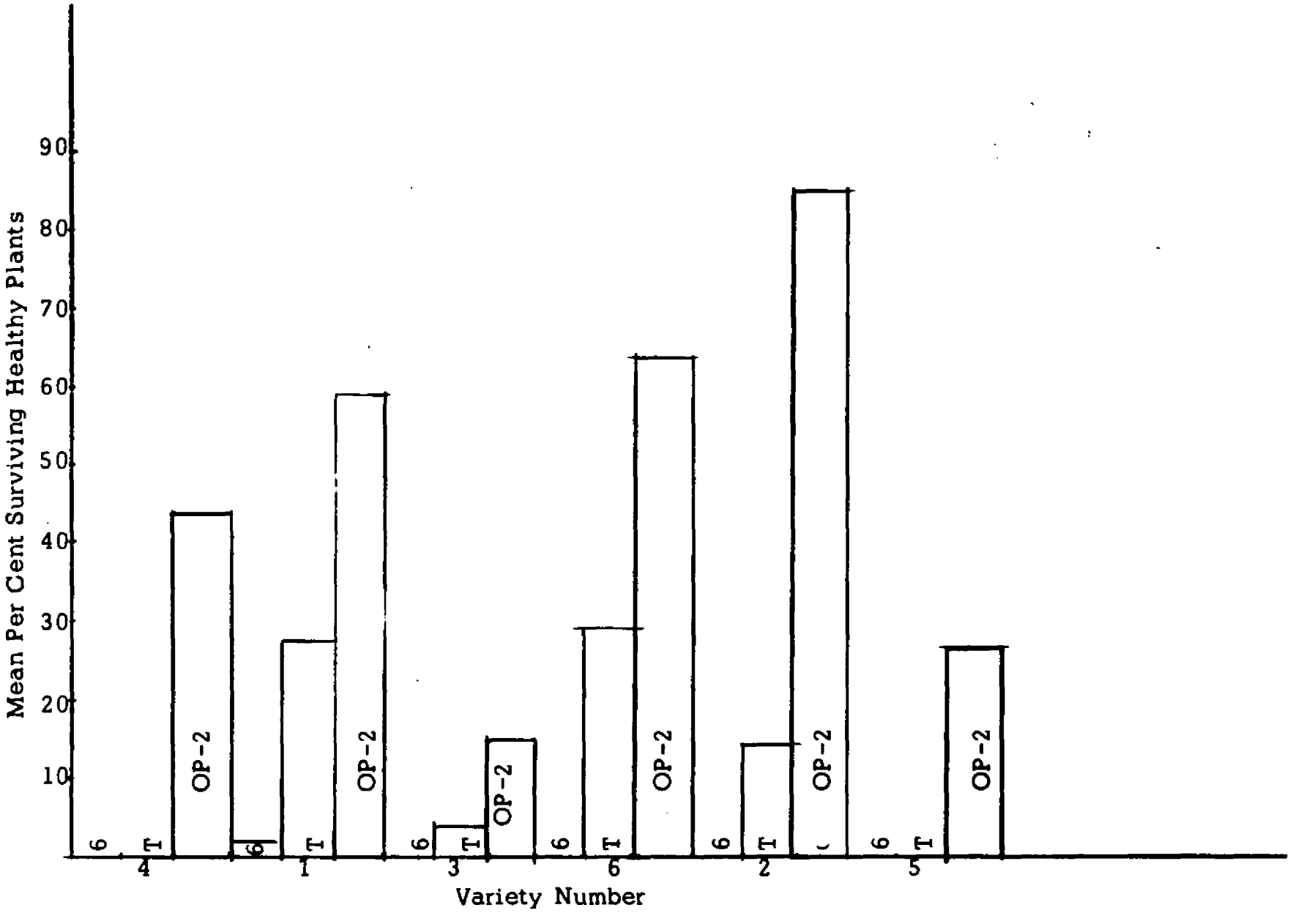

Figure 4. Mean (4 replicates) per cent of surviving healthy plants for Sea Island cotton (G. barbadense) varieties indicated by number grown under controlled temperature (20-24°C) in soil infested with either isolate 6, T, or OP-2 of R. solani.

greenhouse conditions the reaction of 5 Yugoslavian varieties of cotton tested to 3 isolates of R. solani showed these varieties had a survival that varied from 60-100 per cent to isolate OP-2 (Fig. 5). A great variation in their tolerance level was observed under controlled temperature experiments. The survival of healthy plants varied from 6 per cent in CB 30 51 Lot 2 to 40 per cent in CB 30 34. The other lines showed a pattern in between these 2 extremes.

There was no difference in reaction of these varieties to isolate T under greenhouse conditions. All were highly susceptible. No healthy plants survived. Under controlled temperature in the laboratory, there was a noticeable variation in their reaction to this isolate. The percentage of healthy plants varied from 3 per cent in CB 30 51 Lot 1 and 2 to 54 per cen t in CB 30 34, with the other varieties falling in between.

The reaction of these Yugoslavian lines to isolate 6 showed no difference under greenhouse conditions. All were highly susceptible. Under controlled temperature experiments, some lines showed some tolerance. Early Upland had a 12 per cent survival, while CB 30 34 and CB 30 51 showed no survival.

The reaction of the 3 wild species tested to R. solani isolates showed them to be highly susceptible to isolates OP-2 and 6. No plants survived. There was, however, another pattern of reaction to isolate T, in which Gossypium thurberi showed a high tolerance to this isolate. Surviving healthy plants was 83 per cent. G. gossypidies showed 30

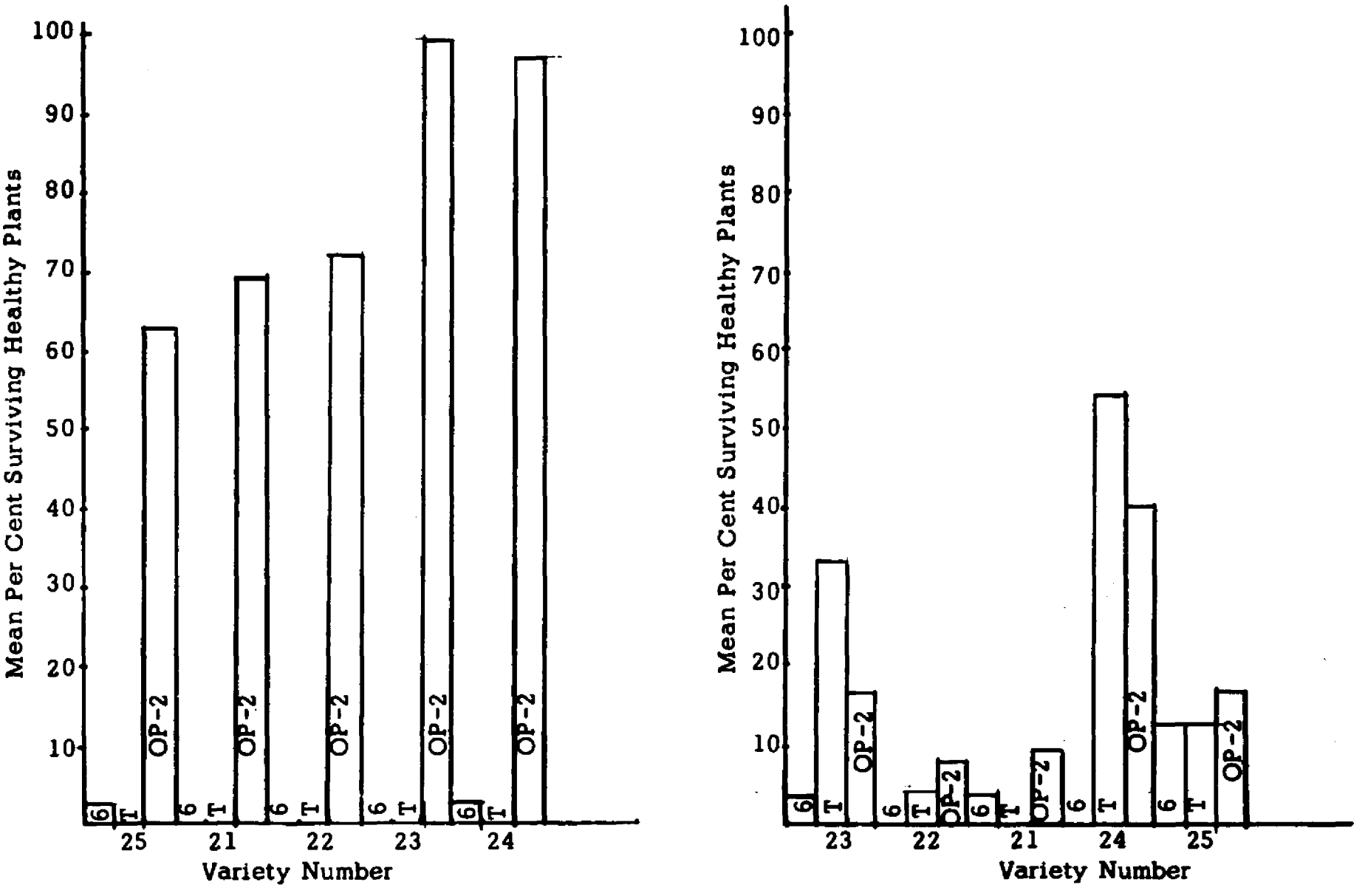

Figure 5. Mean (4 replicates) per cent of surviving healthy plants for Yugoslavian cotton (G. hirsutum) varieties indicated by numbers grown either: under greenhouse conditions (left); or controlled temperature (20-24°C) (right) in soil infested with either isolate 6, T, or OP-2 of R. solani.

per cent survival, while G. ramondi showed no survival. It should be mentioned that the percentage germination was very low (30-40 per cent). Seed of the other 3 wild species did not germinate. The results from tests under field conditions carried on at Northeast Louisiana Branch Experiment Station in 1963 and 1964 and for Red River Valley Station for 1963 are presented (Fig. 6, 7, and 8).

At the Northeast Louisiana Branch Experiment Station there was a great variation in the reaction of the cotton varieties to the seedling disease complex in 1963 and 1964. In 1963 healthy surviving plants varied from 9 per cent in Pima S-1 to 82 per cent in Y 257 (a glandless cotton line). The other of the varieties fell in between.

In 1964, surviving plants varied from 26 per cent in Dixie King to 95 per cent in Menoufi, with 92 per cent in both glandless 1 and CB 3034.

The percentage of healthy plants surviving that year was relatively higher than in 1963.

At the Red River Station, varietal reaction varied from 12 per cent survival in Stoneville 7-A to 86 per cent survival in Menoufi and 82 per cent in CB 3034. The other varieties tested fell in between these 2 extremes.

It appeared that a variety susceptible under greenhouse conditions might appear resistant under field conditions. From these field tests Yugoslavian and glandless lines showed promise for resistance to the cotton seedling disease complex.

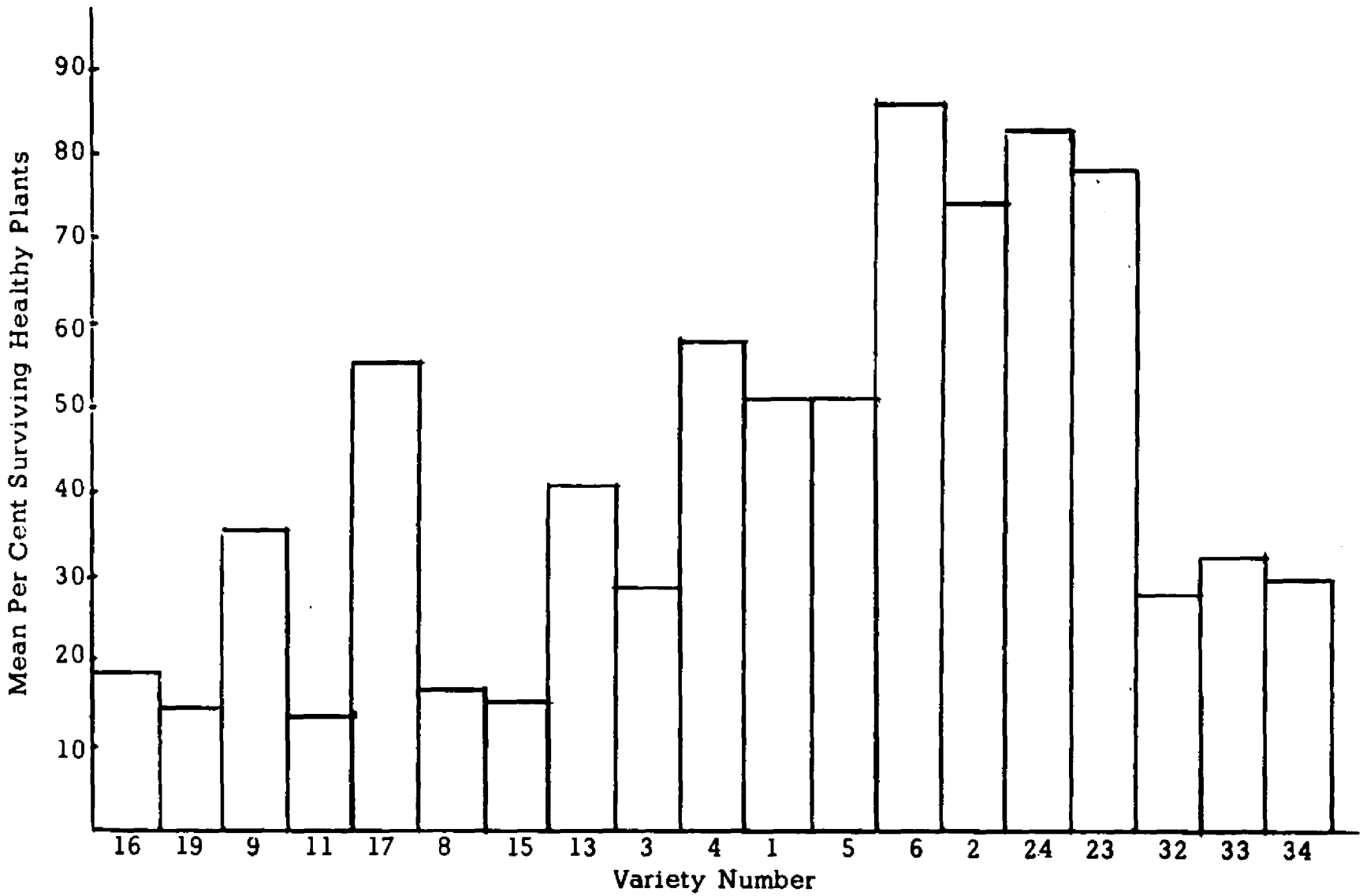

Figure 6. Mean (2 replicates) per cent of surviving healthy plants for different cotton varieties and species indicated by number grown under field conditions at Red River Valley Branch Experiment Station, 1963.

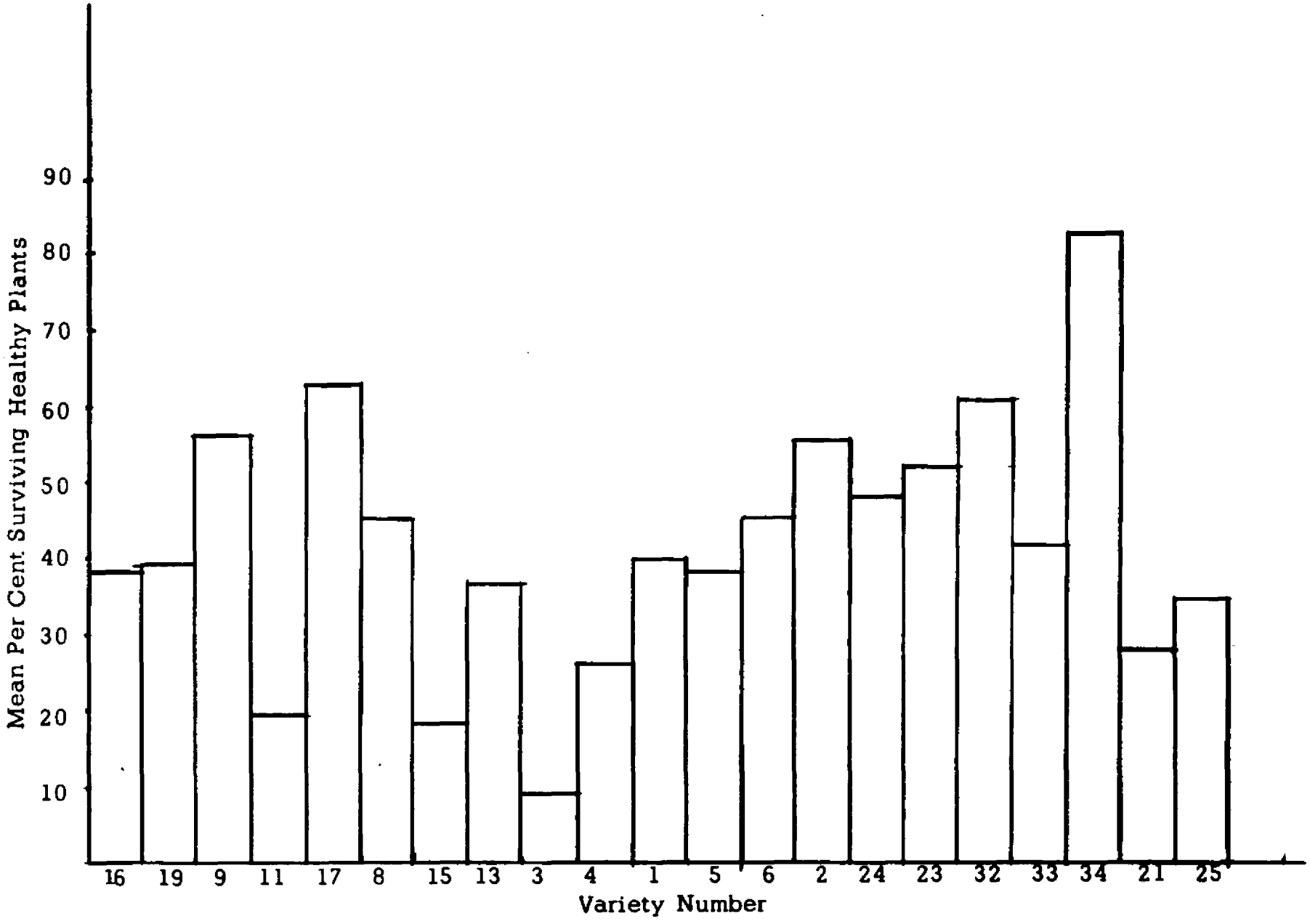

Figure 7. Mean (2 replicates) per cent of surviving healthy plants for different cotton varieties and species indicated by number grown under field conditions of Northeast Louisiana Branch Experiment Station, 1963.

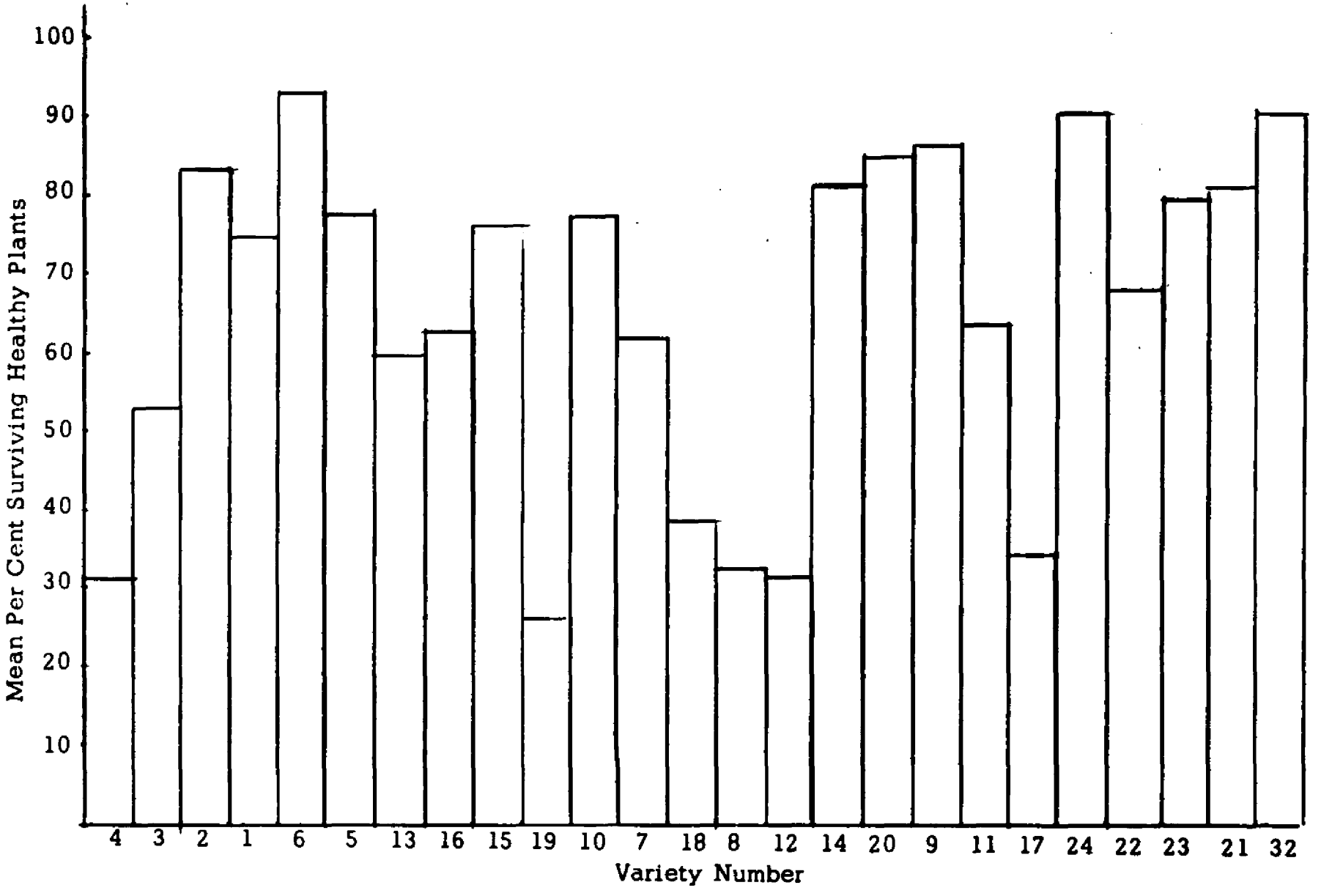

Figure 8. Mean (2 replicates) per cent of surviving healthy plants for different varieties and species of cotton indicated by number grown under field conditions at Northeast Louisiana Branch Station, 1964.

The results of the effect of radiation on the tolerance of cotton lines to the attack of R. solani isolates are given (Fig. 9). A special pattern appeared from results of this test. Lines treated with 500 roetgens appeared to have resistance to the 3 R. solani isolates. A high percentage survival was recorded in these lines when compared with checks. Both above and below this irradiation dosage, the pattern was not evident. These results suggest that further studies should be made using a larger number of seed to establish the effect of irradiation in developing cotton varieties with field resistance.

From the results the question arose as to whether or not this variation in resistance and susceptibility of these cotton varieties, lines, and species is enough to be used as a base for considering physiologic specialization of R. solani on cotton. For this purpose 12 differential varieties of cotton were chosen which showed the greatest variation in reaction.

The level of evaluation was determined from data obtained in the greenhouse. This arbitrary level for differentiating races was set up using the survival percentage in infested flats. Varieties that had a per cent survival of 0-40 were considered as susceptible (-), 50-70 per cent as moderately resistant (+), and 70-100 per cent as resistant (++). It was thought that a variety with a 50 per cent survival under greenhouse conditions would have field resistance, since the greenhouse test was considered more severe than would be encountered under field conditions.

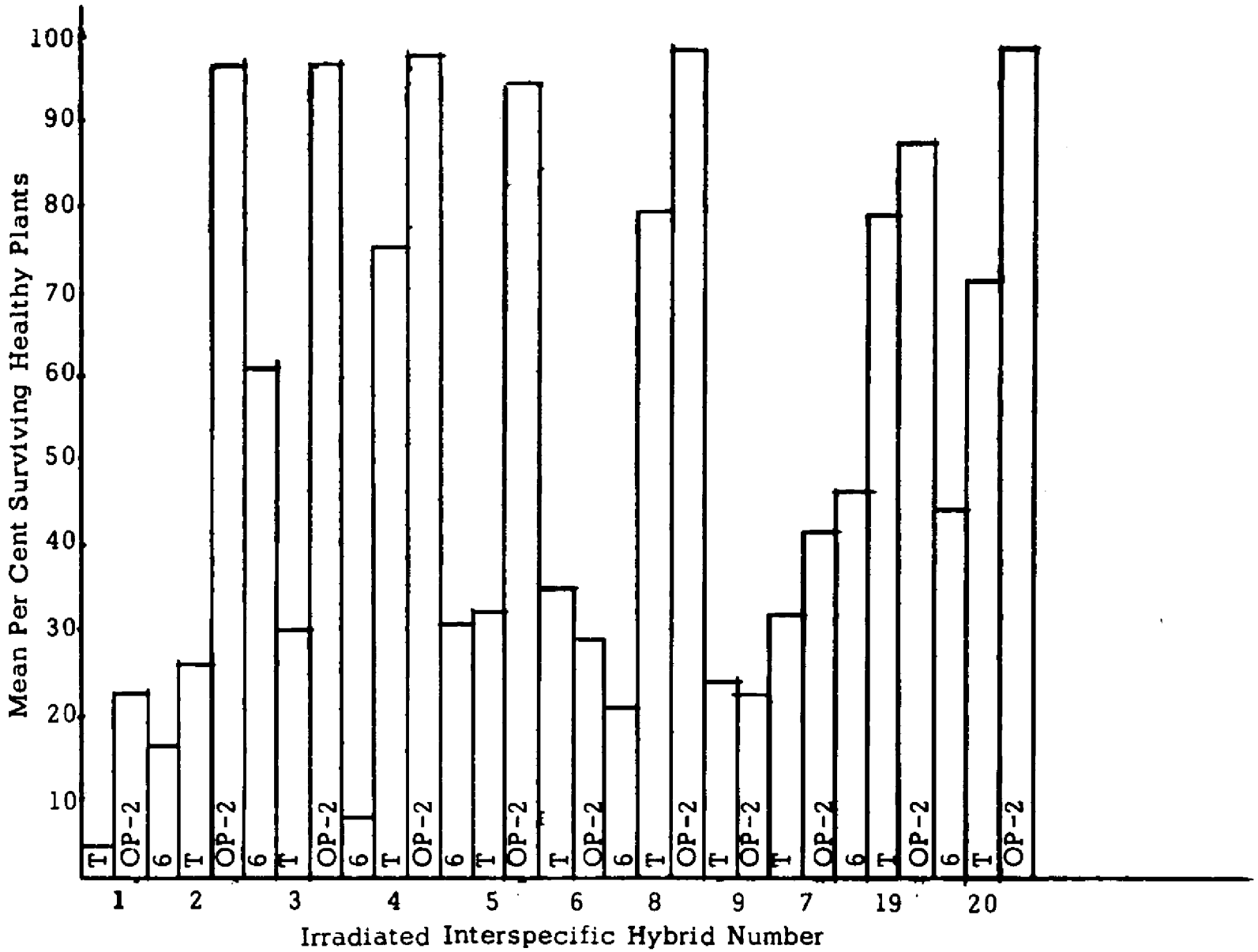

Figure 9. Mean (4 replicates) per cent of surviving healthy plants for interspecific cotton hybrids (G. hirsutum and G. barbadense) irradiated with different dosages of Cobalt 60, indicated by number (see Table 2) and grown under greenhouse conditions in soil infested with either isolate 6, T, or OP-2 of R. solani.

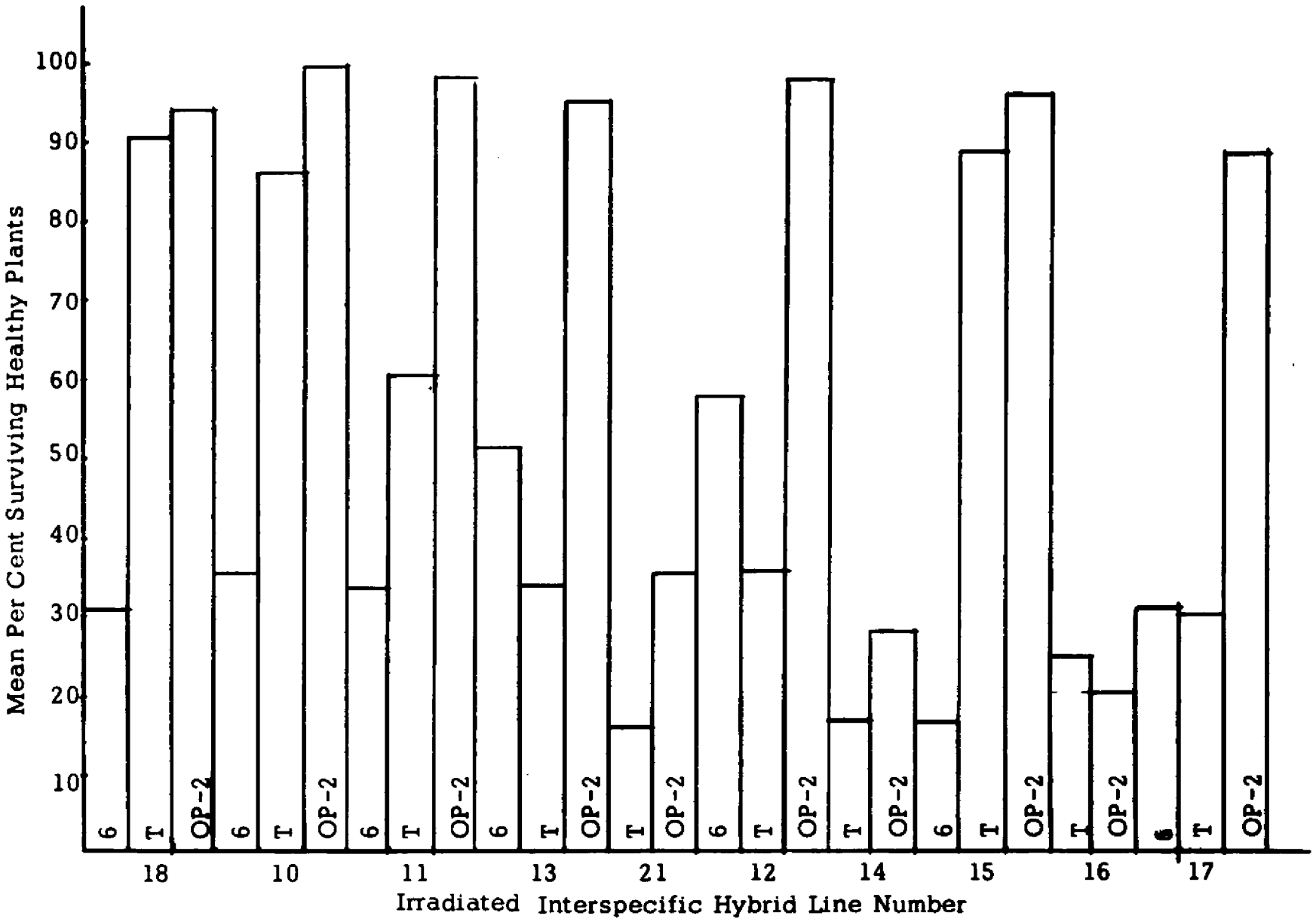

Figure 9. Continued.

The pathogenicity of these races of R. solani on the differential varieties of cotton are presented (Fig. 10 and Table 3). By using these differential varieties, lines and species of cotton, it may be concluded that these 3 isolates of R. solani may be considered as physiologic races. Further studies, however, are necessary. A large number of varieties and a large number of isolates should be tested to better establish the existence of physiologic races in R. solani.

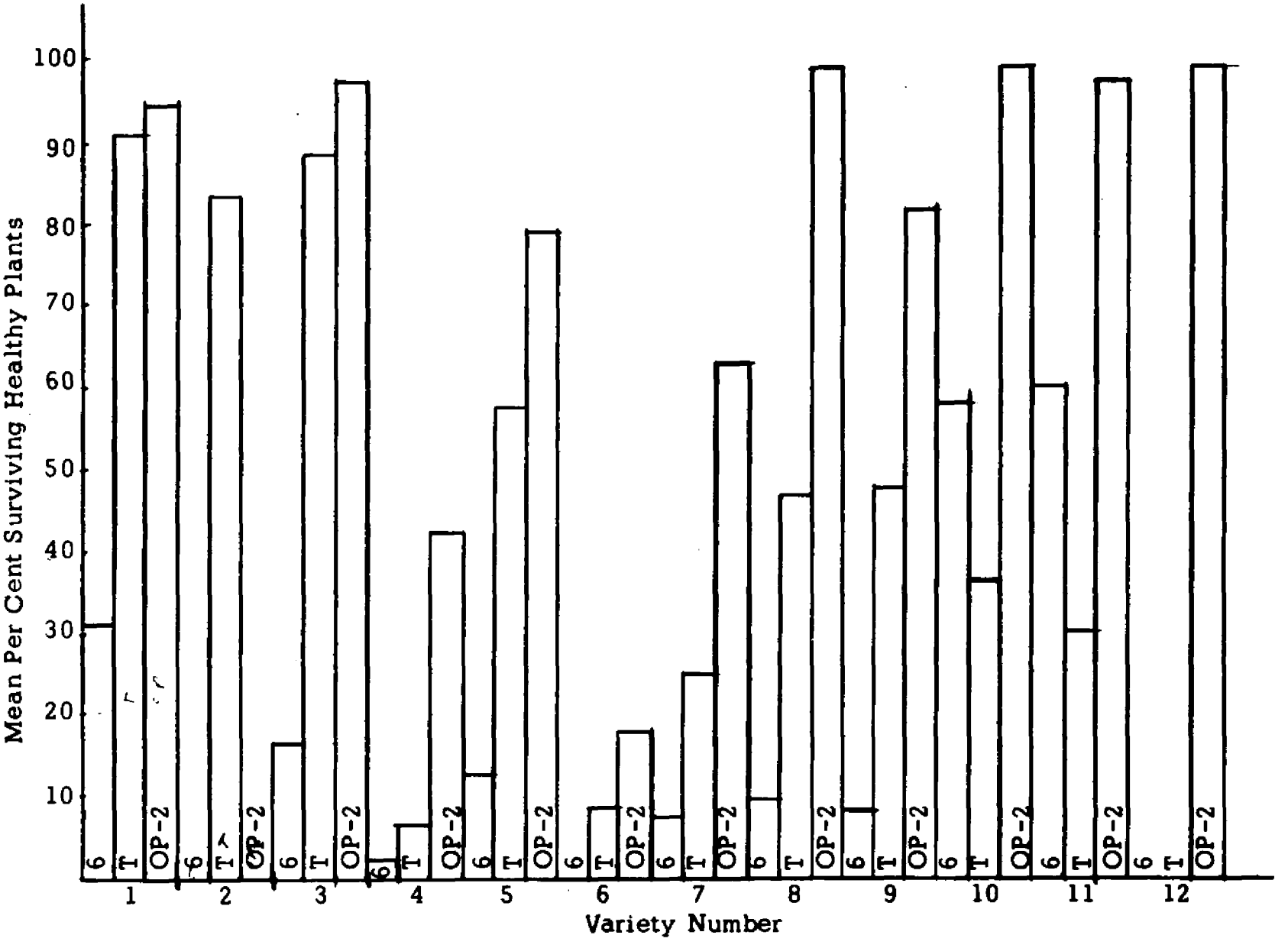

Figure 10. Mean per cent of surviving healthy plants for 12 differential cotton varieties (Table 3) grown under greenhouse conditions in soil infested with either isolate 6, T, or OP-2 of R. solani.

Table 3. The behavior of Rhizoctonia solani isolates on the 12 differential varieties of cotton.

No.	Differential cotton varieties and their species	R. solani isolates T	6	OP-2
1	G. hirsutum x G. barbadense Line 295	++	-	++
2	G. thurberi	++	-	-
3	G. hirsutum x G. barbadense Line 19	++	-	++
4	G. hirsutum Fox 4	-	-	-
5	G. hirsutum DPL smooth leaf	+	-	++
6	G. hirsutum DPL 15	-	-	-
7	G. hirsutum Carolina Queen	-	-	+
8	G. barbadense Amsak	-	-	++
9	G. barbadense Prog. S x P	-	-	++
10	G. hirsutum x G. barbadense Line 319	-	+	++
11	G. hirsutum x G. barbadense Line 257	-	+	++
12	G. hirsutum Yugoslavian Line CB 3035	-	-	++

DISCUSSION

Part I

The phenomenon of physiologic specialization is important from a practical, as well as, a scientific standpoint. Varieties resistant to a given pathogen and developed for certain localities, do not always remain resistant. New physiologic forms may appear at any time and render resistant varieties completely susceptible. There are many pathogenic races of R. solani with wide host ranges. This is significant from the standpoint of disease control, for rotation and sanitation programs would be impractical when many crops are hosts to the same pathogen.

It was observed in this study that the cotton varieties, lines, strains, and species tested varied in their reaction to 3 R. solani isolates. In the Upland group a great variation from resistant to susceptible was observed. The reaction of any one cotton variety to Rhizoctonia infection depended upon virulence of the isolate and environmental conditions. In this study Deltapine smooth leaf, which was resistant under greenhouse conditions to isolate T, showed high susceptibility under controlled temperatures of 20-24°C. Under greenhouse conditions the Upland group appeared more susceptible to R. solani infection than did the Sea Island group.

Results showed that all groups of cotton varieties behaved similarly,

in that there was a great variation in their resistance level to 3 isolates of R. solani.

The resistance of cotton varieties to R. solani infection behaved as any other biological character in that a small number of varieties fell in either susceptible or resistant categories, while the majority were intermediate. The resistance or susceptibility of a new variety was determined by the interaction of host, pathogen and environment. Results of temperature on the reaction of cotton varieties to Rhizoctonia sp. infection agreed with those of Smith (77) and Peturson (54).

Fulton et al. (30) reported that none of the lines tested showed reasonable resistance to R. solani except some cold tolerance varieties from Yugoslavia. Results reported here indicated that there was a considerable resistance to R. solani in some varieties. The virulence of a given isolate was one of the determining factors for the resistance or susceptibility behavior of a given variety.

Some varieties showed a high degree of resistance to isolate OP-2 with almost 100 per cent survival while others were completely susceptible. For isolate T, 83 per cent survival was expressed in G. thurberi and 60 per cent in some Sea Island varieties, while, isolate 6 was highly virulent and all of the varieties, lines, and species were highly susceptible to it. A considerable amount of resistance to isolate 6 was recorded in irradiated lines, which were the result of crosses between Sea Island and Upland cotton varieties. It was suggested that a large number of varieties

from all over the world should be evaluated by inoculation with a large number of R. solani isolates. The varieties, location and season interaction should be considered because of their great effect on the behavior of the varieties to R. solani infection.

The technique used in this study was one of the main reasons for success in expressing such resistance in cotton varieties to infection. Waddle (88) mentioned that some tolerance to R. solani was observed in G. thurberi, and G. arborum but even this was not impressive and may have been a function of a technique rather than material. Conditions under which the work of Fulton and Waddle (30) was done were severe. In this study 10-15 cc of mycelial suspension were applied to the flat at the time of planting to minimize the severity of the pathogen. Even using this technique it was believed that the condition for evaluation was still severe in comparison with natural infection in the field. Some lines that proved to have some tolerance to R. solani under greenhouse conditions may have economical use under field conditions.

Results from evaluation of cotton varieties, lines and strains under field conditions showed that under natural infection some lines proved to have practical and economical resistance to the seedling disease complex. The location and time of evaluation was considered as one of the most important factors in determining the behavior of cotton varieties to infection. It should be kept in mind that the evaluation of varieties should be made for at least 6 years, and at least 6 different locations before reaching a

final decision. Results reported here showed great promise and indicated the possibility of finding a cotton variety practically and economically resistant to R. solani damage. Glandless cotton lines and Yugoslavian strains showed noticeable resistance under field evaluation.

Results using irradiated cotton seed showed considerable tolerance of these lines to the 3 isolates of R. solani in comparison with non-irradiated material. The seed were limited in quantity and these were the results of one year's testing, but it showed a special pattern of behavior which needs further evaluation and study. It may be possible by the use of irradiation to obtain a variety that would have resistance to R. solani under field conditions.

Reaction of cotton varieties, lines and strains to R. solani isolates showed variation from resistant to susceptible. Using the most differential varieties, it was concluded that these isolates of R. solani could be considered as physiologic races. A single isolate varied in its reaction on different cotton varieties, and a single variety varied in its reaction to different isolates. It should be emphasized that these results are considered as a step towards the establishment of physiologic races of R. solani on cotton. A larger number of varieties than tested here should be evaluated against different isolates in an attempt to identify the races. Stakman and Christensen (81) stated that the identification of physiologic races of the pathogenic fungi such as R. solani was very difficult, because the criterion for evaluation is the percentage of plants killed, and there is always great range in this quantitative character.

It was observed that R. solani isolates, made in 1959, and maintained in culture, showed no observable variation in morphology or tolerance level to PCNB (73). They appeared to be very stable.

LITERATURE REVIEW

Part II

Cytological studies made in the past on fungi deal mainly with nuclear behavior in sexual cells during meiosis (51). These studies record occurrence of meiotic and/or mitotic divisions in cells of the plasmodia of Myxomycetes; sporangia and oogonia of Phycomycetes; asci of Ascomycetes and basidia and basidiospores of Basidiomycetes. In contrast to this, behavior of nuclei in somatic cells of fungi has received little attention until recently. Descriptions of somatic nuclear divisions are scarce and nearly always incomplete.

Several reasons can be cited to explain the scarcity of observations of nuclear divisions in the somatic cells of fungi. The first of these cited by a number of investigators is that nuclei in these cells are too tiny to be the object of satisfactory cytological studies (55). Lindegren (41) discussing somatic nuclei in the ascomycetes stated, "The haploid somatic nuclei found in the mycelia, conidia, and perithecial walls are minute densely staining bodies." Pinto-Lopes (55) mentioned that the nucleus has a great variability of structure in different cells of the same thallus. These difficulties led the investigators to look for techniques which would reveal the presence or absence of nuclei. A technique which was good for revealing the presence of nuclei was not equally as good for the study of their structure. Pinto-Lopes (55) stated further that the structure

of the haploid nuclei at resting stage was similar in their constituents and morphological types to those in higher plants. Nuclear constituents were: membrane, nucleoplasm chromonemata and nucleolus.

A second reason may be attributed to the use of haematoxylin as a standard stain for resting and dividing nuclei in somatic cells. It is becoming increasingly apparent that this dye, which has a long history of great usefulness in general cytology and in studies of protozoa, usually failed to reveal the site of the Feulgen-positive matter in nuclei in somatic structures of fungi (57, 62). Though haematoxylin does stain chromatin of nuclei in sexual cells of many fungi it is uninformative when employed to stain the nuclei in somatic cells.

Mode of division in the somatic fungus nuclei

The somatic nuclei differ in their mode of division. Among recent workers on the cytology of fungi, Knox-Davies and Dickson (37) mentioned that cells of vegetative hyphae of Helminthosporium turcicum were generally multinucleate. The interphase nuclei resembled those of higher organisms, with a matrix of thread-like chromatin material surrounding a spherical nucleolus. They mentioned also that nuclear division was rapid and that all nuclei in a cell divided simultaneously. Mitotic figures were observed. El-Ani (26) mentioned that somatic nuclei in hyphae of Hypomyces solani f. cucurbitae divided by ordinary mitosis. He claimed that the size of nucleus varied with width of hyphae. The

wider the hyphae, the larger the nucleus. He mentioned, also, that at prometaphase, a prominent nucleolus frequently was observed.

Cutter (19, 20) mentioned that in vegetative mycelium in sporangia of mucorales, nuclei divide by ordinary mitosis.

Cole and Couch (16) reported that Kabatiella caulivora in some instances, mitotic nuclear divisions within the conidium occurred for periods up to 24 hours without being accompanied by, or production of, a germ tube. During metaphase, the equatorial plate was oriented perpendicularly to the long axis of the conidium with spindle fibers parallel to the long axis. They further observed that chromosomes were evident in the interphase nucleus and were distinct from prophase through metaphase. Their observations of nuclei and cells of various mitotic stages supported in all respects the results of phase contrast examination of living preparations. Colson (18) reported that mycelial cells of Neurospora tetrasperma were multinucleate throughout development and mitotic division took place in nuclei of all spores.

Somers, Wagner and Hsu (79) reported their observations on mitosis in vegetative nuclei of Neurospora crassa. They mentioned that resting nuclei appeared as small, densely stained bodies approximately 1 u in diameter, which in this extremely condensed state, lacked all structural details usually visible in interphase nuclei. They showed that hyphae from cultures several weeks old showed nuclear degeneration and disintegration. Nuclear remains and fragments were of extremely

variable size and shape. Since many of these fragments were within the size limits of chromosomes, it was desirable to avoid examining cultures of this age for evidence of mitosis. Their observations presented evidence for division of vegetative nuclei in N. crassa which was similar to mitosis of somatic nuclei of other organisms.

Cutter (21), reported that somatic nuclei in hyphae and conidia of N. tetrasperma were small and unexpanded. Chromosomes in somatic divisions were rarely more than 1 u in length, but could be counted in properly oriented metaphase figures. Six chromosomes were distinct during the metaphase figure. Ward and Ciurysek (91), provided conclusive evidence that somatic nuclei of N. crassa divided by mitosis in essentially the same way as vegetative nuclei of other organisms.

Hartmann (31), reported that nuclei in vegetative mycelium of Alternaria tenuis divided mitotically. A study of hyphal and conidial nuclei using the Feulgen reaction, indicated that division was mitotic. He mentioned that 5 chromosomes and a spindle apparatus were formed during nuclear division.

Robinow (65), supported the theory of mitotic nuclear division in somatic mycelium of fungi by his studies on the fungus Basidiobolus ranarum. He mentioned that mitosis was accompanied by a temporary coarsening of the organization of cytoplasm and a considerable slowing down of growth rates of cell wall tubes. An interesting feature of its mitosis was the spindle apparatus, which the metaphase chromosomes

generated in the rearranged substance from the former nucleolus. He concluded that the feature of Basidiobolus sp., that appeared most common place to cytologists, was that the nucleus divided by an ordinary form of mitosis.

Turian andCantino (87) showed intranuclear mitosis took place in Blastocladiella sp. and in early prophase nuclei revealed the localization of chromatin in the form of a dense ring around the presumably proteinaceous matrix of the spherical nucleolus. As mitosis proceeded chromatin reorganized in a crescent-shaped form around the still spherical, but enlarged and hypertrophied, nucleolus.

Mitotic stages of vegetative nuclei in Blastocladiella emersonii, as well as in Allomyces sp., did not reveal clear-cut individual chromosomes. Of course, the possibility could not be overlooked that persistence of the nuclear membrane around chromatic material (intranuclear mitosis) might have prevented normal scattering of individual chromosome in these fungi.

Callen (13), reported in Rhizopus sexualis division were intranuclear and mitotic division took place in vegetative nuclei. The prophase was indicated by an increase in size of this mass, which assumed an irregular oval shape, and stained unevenly. It has not been possible to follow the development of the spindle during prophase, but a lighter staining cone-shaped mass has sometimes been observed on one side of the chromatin. This might be regarded as a portion of the spindle.

With the development of metaphase, the spindle assumed a more or less central position over the mass of chromatin.

Olive (50), mentioned that vegetative nuclear divisions have been studied in the spermogonia and in other gametophytic hyphae, as well as in the binucleated sporophytic hyphae of the rusts. The process was essentially the same in all these types of cells, being a mitotic phenomenon. Each nucleus, during the conjugate divisions, acted apparently in an entire independent manner of its associated nucleus. During the earlier stages of the association of the 2 nuclei in the 1 cell, just following the sexual fusion, the mitotic figures were sometimes variously oriented in the cell, bearing no obvious relation to each other. He further mentioned that each nucleus divided by the aid of a centrosome, which was located on the nuclear membrane, and which, in some forms, persisted in the resting stages as a distinct point of polarization of the nuclear contents.

Ward and Ciurysek (90), showed that nuclei in stained preparations of mycelium of an unidentified Basidiomycete, which was the causal agent of snow mold, were shown to divide by mitosis. Typical stages of division, with the exception of early prophase, were clearly distinguished and the haploid chromosome number was shown to be 4.

Hartog (32), described a rudimentary mitosis which he called "transition between a direct and an indirect division." He saw 4 small granular chromosomes, which split longitudinally and then separated. No spindles were reported as being present.

Dowding, and Weijer (25), found that mitosis in Neurospora sp., mycelium was unlike that in higher plants and animals and that there was no spindle, but they have observed that nuclei were filamentous and they divided by splitting longitudinally. They observed the following types of nuclei which they believe to be consecutive stages in mitosis:

1. A network within a spherical membrane.
2. An elongated network free of the membrane.
3. A narrow thread.
4. A thread longitudinally split.
5. Two separate daughter threads.
6. A shortened, thickened filament with distinct chromosomes.
7. A similar filament coiled with a membrane.

All these reports support the theory that somatic nuclei divide by ordinary mitosis as has been observed in higher plants and animals.

There is a group of investigators who support another theory of the mode of division in somatic nuclei. They claim that it does not follow the conventional system of mitosis but was achieved by elongation of the nucleus followed by constriction at its mid-region. At the end of division the extremities pull apart quickly to form the chromatin portions of the 2 sister nuclei.

Robinow (62, 63), has recorded observations on the structure and behavior of the nuclei in spores and growing hyphae of several mucorine fungi. He noted that the living, resting nuclei of Mucor hiemalis and M.

fragilis consisted of a dense central body, the nucleolus, surrounded by a shell of variable shape composed of optically uniform material of low density. These nuclei divided by constriction. One-half of the nucleolus and one-half shell of the low density material passed to each daughter nucleus. All phases of nuclear division could be recognized in fixed and stained preparations. The nucleolus was readily stained by iron alum haematoxylin but was Feulgen-negative. Shells of low density had no marked affinity for haematoxylin or other basic or acid stains but consisted of Feulgen-positive granules and filaments. Robinow (62), postulated that in these elements chromosomes must somehow be contained but was unable to resolve the individual chromosomes. Division of the mass of chromatinic elements was direct and involved neither spindle nor metaphase plate.

In the several strains of Phycomyces blakesleanus studied, Robinow (63), further mentioned that nuclear structure and manner of division were similar to that observed in M. hiemalis and M. fragilis (62). One strain contained usually large nuclei. These nuclei consisted of a shell of tightly packed granules and filaments of chromatin curving around a relatively large nucleolus. The nuclei divide by elongation followed by constriction. The nucleolus divided at the same time and in the same way and one-half of it passed to each sister nucleus. Robinow's detailed observations indicate that nuclei in the vegetative cells of mucorine fungi did not divide in the manner of classical mitosis (62, 63).

Bakerspigel (1, 2, 3, 4, 5, 6, 7, 8), reported on nuclei in somatic cells of 11 species of fungi. He mentioned that the chromatin of vegetative fungal nuclei has no marked affinity for direct nuclear stains regardless of whether the nucleus is at rest or in a state of division. In stained preparations most interdivisional of resting nuclei was composed of a shell or crescent of granular chromatin partially or wholly surrounding a spherical or oval central body. In some nuclei the chromatin was more homogenous than in others. He further mentioned that the nuclear membrane had not been observed surrounding the resting nuclei in any of the stained preparations studied. In living preparations resting nuclei appeared as optically dense central body surrounded by an optically clear area. The nuclear membrane had not been observed surrounding the resting nucleus in any of the living preparations studied. Bakerspigel (1, 2, 3, 4, 5, 6, 7, 8), further mentioned that vegetative nuclear division proceeded in the following manner. The nucleus becomes angular constricts and finally the 2 extremities separated. Individual chromosomes, metaphase plates or spindles has not been observed. Each of the sister nuclei presumably contained equal portions of the original chromatin and central body. In Neurospora sp. (4), and Gelasinospora sp. (5), configurations of chromosome-like bodies has been observed during their division. In none of the Ascomycetes studied by him have individual chromosomes, metaphase plates or spindles been observed. He further mentioned that in contrast to ordinary mitosis vegetative fungal nuclei divide rapidly, usually within 4-6 min. He concluded that these vegetative fungal nuclei presumably

contain chromosomes though they do not divide by ordinary mitosis. The final distribution of these chromosomes, previously segregated in the resting nucleus.

Lindegren and Rumann (41) mentioned that the haploid somatic nuclei found in the mycelia, conidia and perithecial walls of N. crassa were minute, densely staining bodies, Reticulate structure, nucleoli and nuclear membrane were found only in stages having an obvious relation to sexual reproduction, whether in perithecia or shortly before their formation. Lindegren, et al. (41) never found spindles except in the ascus. Somatic haploid nuclei were merely dense, tiny globules containing chromatin. In a sexual tissue of many ascomycetes, nuclei did not appear to be nucleolate, reticulate or vascular nor was it possible to find evidence for the view that they divide by mitosis.

Burgeff (12), described nuclei of Phycomyces blakesleeanus as minute clusters or chromatin particles lacking both the nucleolus and a nuclear membrane. The clusters were said to divide by falling apart into small clusters.

Trow (86) mentioned that nuclei in Saprolegnia sp. were bounded by a nuclear wall and possessed one central chromosome of spongy texture. The space between the nuclear wall and chromosome was occupied by a nucleo-hyaloplasm, which was traversed by 5 threads. The nucleus underwent direct division in the zoospore and mycelium. Smith (75) noticed that in examining a long hyphae of the fungis, Saprolegnia sp. nuclei were

neither uniform in shape nor size, but there was a gradual transition in shape from a spherical form to a long torpedo-like form in the lower parts of the thallus. He further studied that the nuclei in the vegetative hyphae were found to divide by direct division.

Sansome (70) reported that chromosomes in vegetative hyphae of Pythium debaryanum did not condense and form a metaphase plate as did chromosomes in a typical mitosis and as did the meiotic chromosomes.

Robinow (64) mentioned that Mucor fragilis nuclei seemed to divide directly by elongation and constriction. The nucleolus divided at the same time and in the same way. Mucor sp. chromosomes were not normally visible as separate entities but could be seen clearly in dividing somatic nuclei of Allomyces arbuscula. In contrast to Mucor sp., the nucleolus of Allomyces sp. was dissolved during division. Metaphase plates and spindles were not encountered.

Nuclear movement

There are several reports in which the investigators mentioned nuclear streaming and the passage of nuclei through septal pores as a matter of variability.

Dowding (24) mentioned that under some conditions large number of nuclei of Gelasinospora tetrasperma travel through mycelium for long distances, passing from one cell into the next via a septal pore. Nuclei were carried by the streaming cytoplasm at speeds as high as 40 mm per hour. Under other conditions, moving cytoplasm left all nuclei behind, fixed in the thin cytoplasmic lining of the cell wall.

Dowding and Bakerspigel (23) observed that nuclei of G. tetrasperma traveled out of a mycelium of one sex and into one of opposite sex as rapidly as 10.5 mm per hour. Nuclei migrated from cell to cell in a protoplasmic strand, running through the center of the hyphae and had been observed traveling through their own mycelium. Nuclei have been seen passing from cell through the pore in the transverse septum into the next cell. The shape and size of the nucleus fluctuated. Spherical nuclei may change to narrow, elongated sinuous bodies and then resume their spherical shape. The nuclei migrated in either an expanded, contracted, or elongated form.

Dowding and Buller (22) reported that migrating nuclei have been found to travel through a mycelium of G. tetrasperma at a speed of 4 to 5 mm per hour and that light influences migration of the nuclei. In mating experiments it was found that the nuclei move from a darkened mycelium of one sex toward and into an illuminated part of a mycelium of the opposite sex. Migrating nuclei move from cell to cell via the minute central pore in the transverse septum. They further mentioned that in G. tetrasperma and other Pyremomycetes, columns of cytoplasm were constantly flowing towards tips of the growing hyphae. Nuclei of older cells were not carried away in the stream of cytoplasm but remained anchored to their fixed layer of cytoplasm that was passed along the cell wall.

It has been shown by Buller (22) that in the Pyrenomycetes, Discomycetes, and Hymenomycetes, as hyphae alongates, cytoplasm

was constantly flowing toward the growing point. Thus growth of the hyphae was in part due to the extension of cell wall at hyphal tip, and in part due to the newly created space in the terminal cell of the hyphae which was filled constantly by the flowing cytoplasm.

Snider and Raper (78) reported nuclear migration in the Basidiomycete Schizophyllum commune. The effective rate of migration was at least several times faster than hyphal tip growth under the condition studied.

Welsford (92) noticed nuclear migrations in Phragmidium violaceum. He noticed the migration of a vegetative nucleus to a fertile cell. The size of the pore through which the nucleus passes was very variable, sometimes being as much as 3 u in width. He further observed that layer of more or less empty cells occurred immediately below the binuclear fertile cells, and was made up of these cells from which the nuclei had migrated.

Cytological studies on the somatic nuclei of R. solani and its sexual stage Pellicularia

Olive (51), in his comprehensive review of the structure and behavior of fungal nuclei, suggested that the typical life history among the Hymenomycetes involved production, by germinating basidiospores, of monokaryotic mycelium which had uninucleate cells and lacked clamp connections. Most of these fungi were heterothallic and the dikaryotic mycelium with clamp connections and binucleate cells were produced by hyphal anastomoses between compatible monokaryotic mycelia. Karyogamy and meiosis occurred in the basidia, which then developed 4 uninucleate basidiospores.

Saksena (68) studied the nuclear structure and division in the mycelium and basidiospores of Ceratobasidium praticolum (Pellicularia practicola) in both living and differently stained preparations. Cells of the mycelium were multinucleate. Nuclei consisted of a Feulgen-negative nucleolus surrounded by granular Feulgen-positive chromatin. He further noticed that in a nucleus preparing to divide, that the nucleolus became progressively smaller and finally disappeared. Chromatin elongated becoming bar-like and later constricted at the mid-region. New nucleoli appeared in 2 attached portions of the constricted chromatin. At the end of division the 2 portions pulled apart to form 2 sister nuclei. Classic mitotic division as it occurred in higher organisms was not seen. Chromosomal filaments were not discovered in nuclei of basidiospores or of mycelium.

Saksena (67) studied, also, the nuclear phenomena in the basidium of C. praticolum. He reported that cells of vegetative hyphae were multinucleate and those of the hymenia were binucleate. Before fusion, the 2 nuclei in the young basidium became closely adjacent and their chromosomes were differentiated as elongated threads in 2 distinct groups. After pairing, the diploid nucleus underwent meiosis. He further mentioned that the haploid chromosome number was 6 and the second meiotic division was equational. The spindle axis during both these divisions may have been longitudinal, transverse or oblique.

The mature basidum had 4 nuclei, which migrated through sterigmata into the basidiospores. He observed, also, that most of the basidiospores

were uninucleate when young, but became binucleate at maturity. The basidiospores germinated by repetition or directly by a germ tube. In either case coenocytic growth was produced which upon further development, was divided into multinucleate hyphal cells by the formation of cross septa.

Flentji, Stretton, and Hawn (28), in their studies of the nuclear distribution and behavior throughout the life cycles of Thanatephorus sp., reported that in the vegetative phase, young cells contained 4 to 12 nuclei. They reported that nuclear division in the vegetative cells was found to be conjugate, followed by an even segregation of the daughter nuclei. Frequent malfunction of the conjugate division resulting in uneven segregation of the daughter nuclei was almost certainly the reason for different numbers of nuclei in successive cells of young hyphae. No nuclear migration through septa was observed. They observed further that in older hyphae secondary septa formed without nuclear division, resulting in reduced number of nuclei per cell.

The change from vegetative to reproductive phase was associated with septation of hyphae cutting off cells with only 2 nuclei. In the basidia, karyogamy and meiosis occurred, resulting in 4 haploid nuclei which migrated through the 4 sterigmata to form 4 uninucleate spores. Aberrations also occurred in the reproductive phase, 3 nuclei instead of 2 were sometimes included initially in the basidium or 2 nuclei sometimes migrated from the basidium into 1 spore. Flentje, et al. (28) continued in

his observations mentioning that in the nuclei of the vegetative hyphae no evidence was found to support the suggestion by Saksena (67, 68) that these nuclei divide directly.

Fukano (29) showed that the vegetative mycelium in the host tissue and on media of Hypochnus sasakii consisted of multinucleated cells, ranging from 3 to 23, and mostly 6 to 10 nuclei in each cell. He mentioned, also, that nuclei divided conjugately at one point in the cell, where the septum was provided without a clamp connection, thus equal number of nuclei being resulted in 2 daughter cells. Consequently unequal number of nuclei resulted, which he interpreted as being due to nuclear migration at hyphal fusion. The sclerotium contain 6 to 8 nuclei in each cell. The fruiting hyphae consisted of binucleated cells which were produced from multinucleated cells of vegetative mycelia. Basidia were produced in abundance directly from the branches of such fruiting hyphae. They were clavate and produced 4 sterigmata, each of which produced an obovate basidiospore. During development of basidia, 2 nuclei conjugated and then divided twice. Thus, resulting in 4 nuclei, which migrated into 4 spores. The basidiospores were uninucleate in the beginning, but gradually became multinucleate at the division of the nucleus in germination and further development. Usually the mycelial thus formed contain 4 to 6 nuclei. The fungus was homothallic.

Sanford (69) mentioned that the number of nuclei in the hyphal tip cells of R. solani varied from 2 to 15, with the majority being from 4 to 8.

In Y-type cells the number of nuclei varied from 4 to 25, the majority being 4 to 15. The number in nonbranched-type cells varied from 3 to 19, the majority being 6 to 11. He further reported that migration of nuclei through septal pores, in the direction of growth was observed in vivo. Differences in the number of nuclei in the various cells was normal condition of this fungus. The migration of nuclei through the septal pores was given as one of the possible causes of this variability.

Hawn and Vanterpool (33) reported that there appeared to be 12 chromosomes in the diploid nucleus of the basidium of Pellicularia filamentosa. Mature basidiospores were predominantly uninucleate, occasionally a binucleate basidiospore was observed. They mentioned, also, that stained preparations of the mycelium showed that terminal cells of hyphae were multinucleate, and in old cells, binucleate. The means by which the mycelium becomes binucleate is still not known. They did not find any evidence that such a state could be brought about by migration of nuclei through the central pores of the septa. They believed that the cells of the mycelium became binucleate as a result of new septa cutting off pairs of nuclei and with the nuclear division in the terminal cell keeping it in a coenocytic state as long as the substratum supported new growth. Müller (48) mentioned that R. solani was homothallic and formed no clamp-connections, though anastomoses may be produced in abundance. The vegetative mycelium had an indefinite number of nuclei in the cells, but the latter became binucleate in the hymenial layer. The 2 nuclei fuse in the basidium, and the fused nucleus then divide into 4. The

basidiospores, when mature, usually contain 2 nuclei, derived, presumably, from the division of the single nucleus which each spore received from the basidium. Further division may have occurred before or after the spore germinates.

Ultrastructure of R. solani

Bracker and Butler (10) mentioned that the electron microscope revealed the septum in hyphae of R. solani as a complex structure. The cross wall was composed of 2 plates, each composed of lamellae. Aging was accompanied by increase in thickness of the cross wall and a number of lamellae. Near the center of the septum, surrounding the septal pore, was an annular swelling which appears structurally and texturally different from the lamellar cross wall.

As the hypha developed, septal swelling increased in size, reaching a diameter of over 2 u. The swelling grew in a radial direction, away from the central axis of the hypha and, thus, did not close the septal pore. When the mycelium became old, the septal pore became occluded with electron-dense material and protoplasmic continuity between cells was lost.

The ectoplast was continuous from cell to cell in actively growing cultures where it formed the boundaries of the septal swelling. The endoplasmic reticulum lay close to the septum where it was found parallel to the cross wall on both sides. At the center of the septum the endoplasmic

reticulum diverged from this position to form a thickened, porous, electron-dense cap which covered the septal swelling and septal pore.

The complexity of the septal apparatus did not appear to prohibit the flow of protoplasm from one cell to another.

In 1964 Bracker and Butler (11) reported that the diameter of the septal pore increased during protoplasmic streaming. Despite its complex structure, the septum is well adapted to permit mass flow of protoplasm from cell to cell. Discontinuity of membranes and plasticity of organelles expedite protoplasmic movement through the septal pore.

From the previous information available about R. solani, there appeared clearly that little information was available on the cytology of this important pathogen. It appeared that there was a contradiction in the literature concerning the mode of division on the somatic nuclei of fungi. Different investigators have reported different modesof division on the somatic nuclei of the same fungus. All the reports about R. solani did not handle the problem of the mode of division on the somatic nuclei. This study will be worthy for those investigators who are trying to reach to the origin and evolution of fungi. The objectives of the study concerning this part of the manuscript were:

1. To determine the nuclear condition of the cells of R. solani in living and stained preparations.

2. To determine the mode of division of the somatic nuclei of this fungus in living and stained preparations.

3. To show the haploid chromosome number in the somatic nuclei.

4. To reveal the ultrastructure of this sterile fungus under the electron microscope especially, the structure of the hyphal cell wall, the presence or absence of a nuclear membrane, and its structure, and the structure of other constituents in the cell.

MATERIALS AND METHODS

Part II

For studying the nuclear condition and the mode of division in the somatic mycelium of R. solani, Sinclair's isolate T (74), which has been maintained in pure culture since 1959, was used.

The fungus was grown and maintained on potato sucrose agar (PSA) prepared by standard methods using 20 g of sucrose, 17 g of agar and broth from 200 g of autoclaved, peeled Irish potatoes in 1000 cc of distilled water. After 2 to 3 days incubation at room temperature (22-32°C), young hyphae at the margins of the colonies were removed, killed and fixed in a killing and fixing solution. Several fixatives have been tried using different times of exposure. These were:

1. Farmer's fluid (36, 71), freshly mixed, prepared as follows:

95% Ethyl alcohol (or absolute)	3 parts
Glacial acetic acid	1 part

2. Carony's fluids (36, 71), freshly mixed using:

	A	and	B
95% Ethyl alcohol or absolute	6 parts		6 parts
Chloroform	3 parts		1 part
Glacial acetic acid	1 part		3 parts

3. Newcomer's (49) solution composed of:

Isopropyl alcohol	6 parts
Propionic acid	3 parts
Petroleum ether	1 part
Acetone	1 part
Dioxane	1 part

Newcomer's solution was found to be the best killing and fixing agent after 18 hours exposure. Fixed hyphae were removed, washed first in 95 per cent ethyl alcohol, then 70 per cent ethyl alcohol, followed by washing in distilled water. Hyphae were then hydrolyzed in N HCl for 5 min at room temperature, then in fresh N HCl for 6 to 8 min at 60°C to hydrolyze the interfering RNA in the cell. Hyphae then were washed in 5 changes of distilled water. In the last change hyphae remained for 2 to 3 min to swell chromosomes (93).

Several nuclear stains were tried:

1. Modification of Einarson's formula of Gallocyanin was prepared as follows (17):

Gallocyanin	1.5 g
Chromealum	5.0 g
Distilled water	100 ml

The chrome alum was mixed with the water and heated to boiling. The Gallocyanin was added and boiled for about 5 min. Then it was filtered while hot and stored in a dark bottle. It should be prepared every 2 weeks to be fresh.

2. The Aceto Orcein was prepared as follows (38):

Orcein	1.0 g
Glacial acetic acid	45 ml
Distilled water	55 ml

The Orcein was dissolved in hot glacial acetic acid and then diluted, when cool, with 55 ml of distilled water.

3. Conventional Iron Alum hematoxylin stain (36, 71) was used.

4. A fourth stain was aceto carmine prepared by a modification of the procedure described by Smith (74).

Carmine	0.5 g
45% glacial acetic acid	100 ml

The carmine was fluxed with glacial acetic acid for 2-3 hours, cooled and filtered.

Haematoxylin was used by Sanford (69), Hawn and Vanterpool (33) and Muller (48) to stain nuclei and chromosomes of R. solani. All the stains tried were inferior to well-ripened aceto carmine. This stain showed a better differentiation of the chromosomes and nuclei than did the other 3. The optimum time for staining was found to be 6 to 7 min. After staining hyphae were placed on clean, flamed microscope slides. Mycelium was teased apart with sterile needles, heated gently over a flame and covered with a clean coverslip. After cooling, pressure was applied to the coverslip to crush the mycelium and separate the chromosomes. Excess stain was removed and edges of the coverslips were sealed with a sealing agent which is composed of (76):

Distilled water	90 ml
Glycerin	16 ml
Gum arabic	50 g
Chlorol hydrate	100 g

The seal was applied along the edges of the coverslip with either a medicine dropper or camel hair brush. This sealing agent appeared to be very satisfactory and hardened upon exposure to air.

It was found that differentiation of the nuclei and chromosomes improved after storage in the refrigerator for 10 to 15 days (76). Fungus mycelium was kept in good condition up to a year under refrigeration. After this period mycelium and chromosomes began to deteriorate.

Microscopic studies and photomicrographs were made using a Bausch and Lomb light microscope, and Kodak panatomic X film. The light source was a Bausch and Lomb lamp Model PR 27.

Examination of the living mycelium

In order to examine the nuclei of living mycelia with a phase contrast microscope, 1 of 2 procedures as described by Bakerspigel (2) for preparing the material under examination, was used. For the first, a minute inoculum from a rapidly growing culture of R. solani, isolate T, was placed on a coverslip in a small drop of sterile PSB. The coverslip was placed over water to form a Van Tiegham cell and incubated at 24°C for less than 24 hr. For microscopic examination of nuclei, the coverslip, which had been ringed with vaseline, was removed and applied to a glass

slide so that the vaselined rim of the coverslip sealed the mount. This technique was not satisfactory for this study.

The second technique used by Bakerspigel (1) showed to be the best. Small strips of cellophane (1 x 1.5 cm) were sterilized in the autoclave. These sterilized cellophane strips were placed on PSA medium inoculated with isolate T of R. solani and incubated at 24°C for 4 to 5 days. When sufficient mycelial growth was obtained, a cellophane culture was stripped from the agar and immediately placed in a few drops of PSB on a clean, flamed microscope slide. A coverslip was then placed over the preparation, sealed with the sealing agent (76) leaving one corner open for air. This type of preparation was found to be satisfactory for the study of nuclei in the hyphae since the cellophane adhered fairly closely to the undersurface of the coverslip. The slides were examined under a Bausch and Lomb phase contrast microscope.

For examining nuclei and ultrastructure of R. solani, electron microscope studies were conducted. The procedure used for fixing and sectioning for the electron microscope was as follows:

Isolate T of R. solani was grown in sterile PSB for about 4 to 5 days. Mycelial mats were removed from the media with sterile forceps and placed in small vials. Mycelia were fixed with 2 per cent unbuffered KM_nO_4 for 6 hours. The cells were centrifuged, then washed twice with distilled water. The cells were dehydrated as follows:

1.	Placed in 25% alcohol for	15 min
	Centrifuge for	5 min
2.	50% alcohol for	15 min
	Centrifuge for	5 min
3.	70% alcohol for	15 min
	Centrifuge for	5 min
4.	90% alcohol for	15 min
	Centrifuge for	5 min
5.	Absolute alcohol for	15 min
	Centrifuge for	5 min

Cells were washed twice in propylene oxide for 15 min each and centrifuged between each washing. Mycelia were then soaked in 1.1 by volume propylene oxide and maraglas mixture for 30 min. The maraglas mixture was prepared by mixing:

34 ml Maraglas	68%
10 ml Cardolite	20%
5 ml Dibutyl phthalate	10%
1 ml Benzyl dimethyl amine	2%

After this the material was centrifuged for 5 min, placed in maraglas mixture for 1 hr, centrifuged, poured out and new maraglas added. The specimen was placed in a refrigerator at $10^{o}C$ for 12 hr. Then the maraglas was centrifuged and poured off. The material was placed in predried capsules, spun out, and fresh maraglas added to the capsules. The

capsules then were placed in a vacuum oven at about 60°C for 48 hr. in plastic capsules. After this blocks were trimmed and ready for ultramicrotome. The microtome used was Porter-Blum MT-1 using a glass knife. Sections were examined under the RCA EMU-2B electron microscope.

RESULTS

Part II

Nuclei and chromosomes in somatic hyphae of R. solani

Stained preparations of somatic hyphae of R. solani were examined with a Bausch and Lomb light microscope.

The average width of 100 measured somatic hyphae was 7.0 u. Hyphae were multinucleate and the number of nuclei per cell varied widely. In hyphal tip cells the number of nuclei was found to be as high as 15 (Fig. 11). Hyphal tip cells always were filled with chromatic material. In older cells, however, the number of nuclei was few. Often the nuclei were compressed to the inner walls of hyphal cells in older hyphae (Fig. 12). In any strand of mycelium the number of nuclei per cell decreased the further the cell was from the hyphal tip. Some older cells, far back from the hyphal tip, appeared to be free of nuclei.

Resting nuclei appeared intensely stained, small, spherical bodies with definite borders suggesting the presence of a nuclear membrane (Fig. 13, 17), and were typical of the majority of nuclei in old cultures of the fungus. The average diameter of 100 measured nuclei in the resting stage was 1.6 to 1.7 u in diameter. Dividing nuclei were larger with a diameter up to 3.0 u at metaphase stage (Fig. 14, 15, 16).

Because of the peripheral growth habit of R. solani, the ratio of resting nuclei to dividing nuclei tended to be high and increased with age

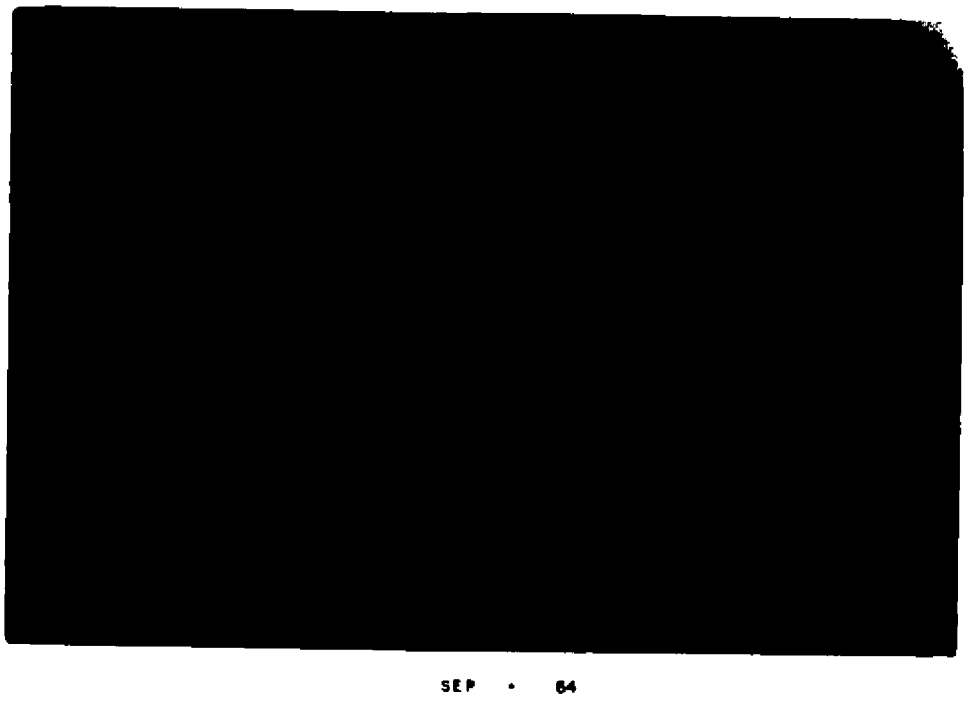

Figure 11. Fixed and stained hyphal tip cell of somatic hypha of R. solani showing multinucleate condition. (X 1000).

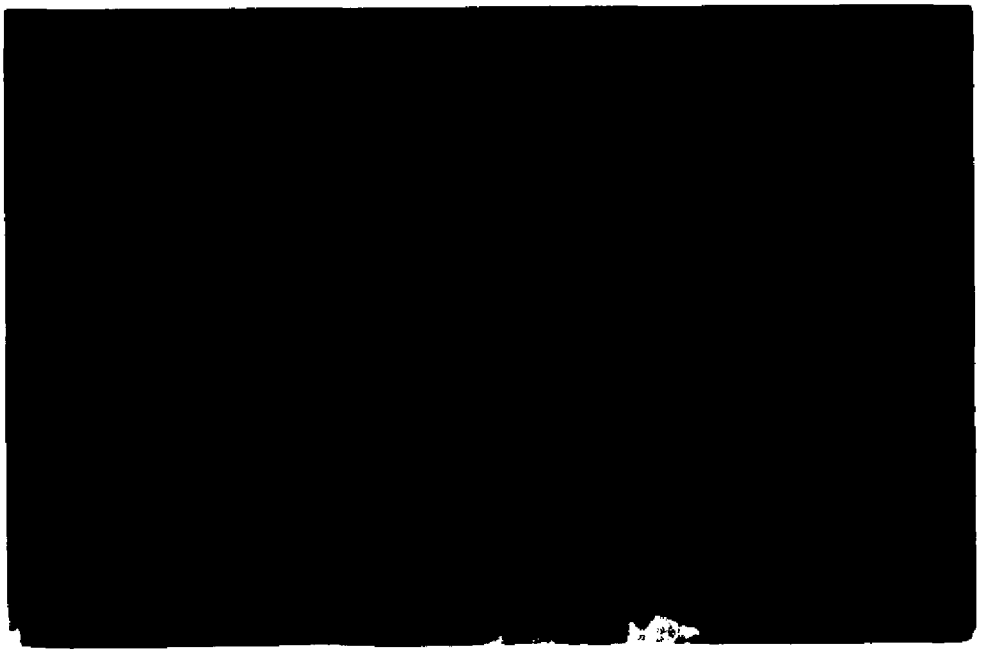

Figure 12. Nuclei in somatic hyphae of R. solani showing nuclei compressed to cell walls in old cells of mycelium. (X 1000).

Figure 13. Hyphal tip cells and hyphal cells of somatic hyphae of R. solani showing resting nuclei. (X 1000).

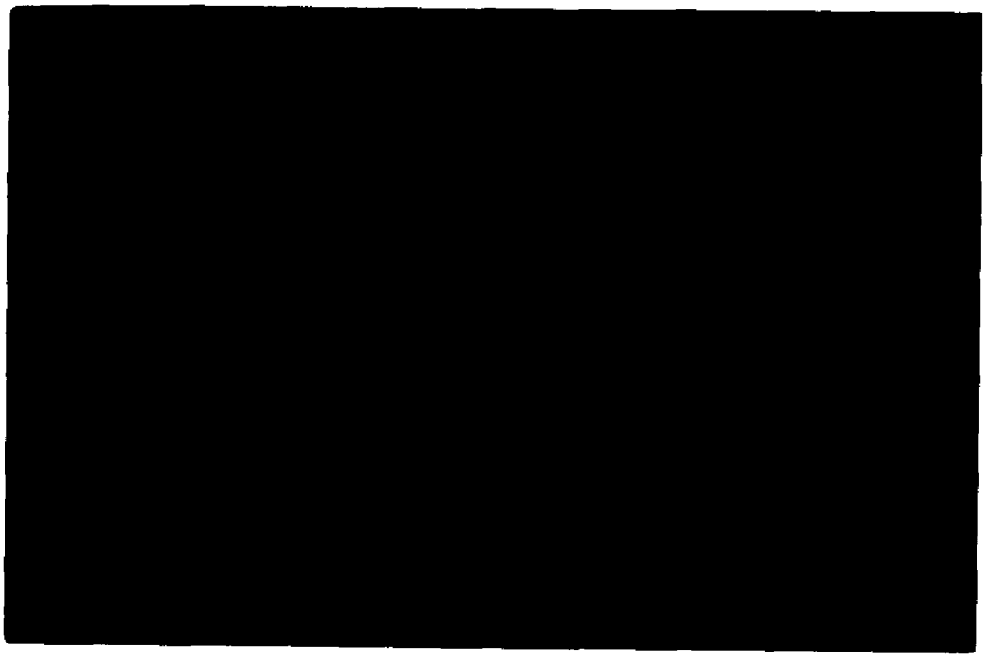

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Figure 14. Nuclei in somatic hyphae of R. solani in metaphase. Above: polar view showing 4 distinct chromosomes (see arrow). Below: side view showing 4 distinct chromosomes (see arrow). (X 1200).

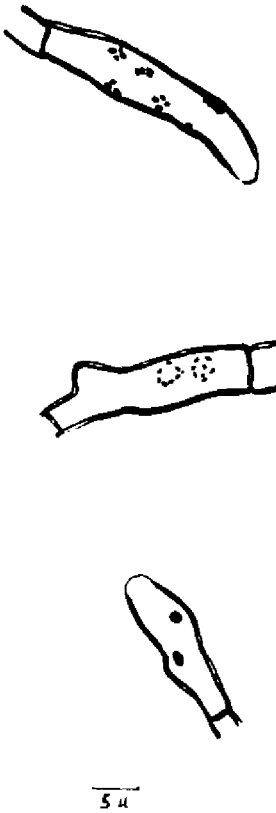

Figure 15. Camera lucida drawings showing nuclei in somatic hyphae of R. solani in metaphase: Top, showing metaphase plates and 4 chromosomes; middle, showing anaphase; and botton, showing resting nuclei.

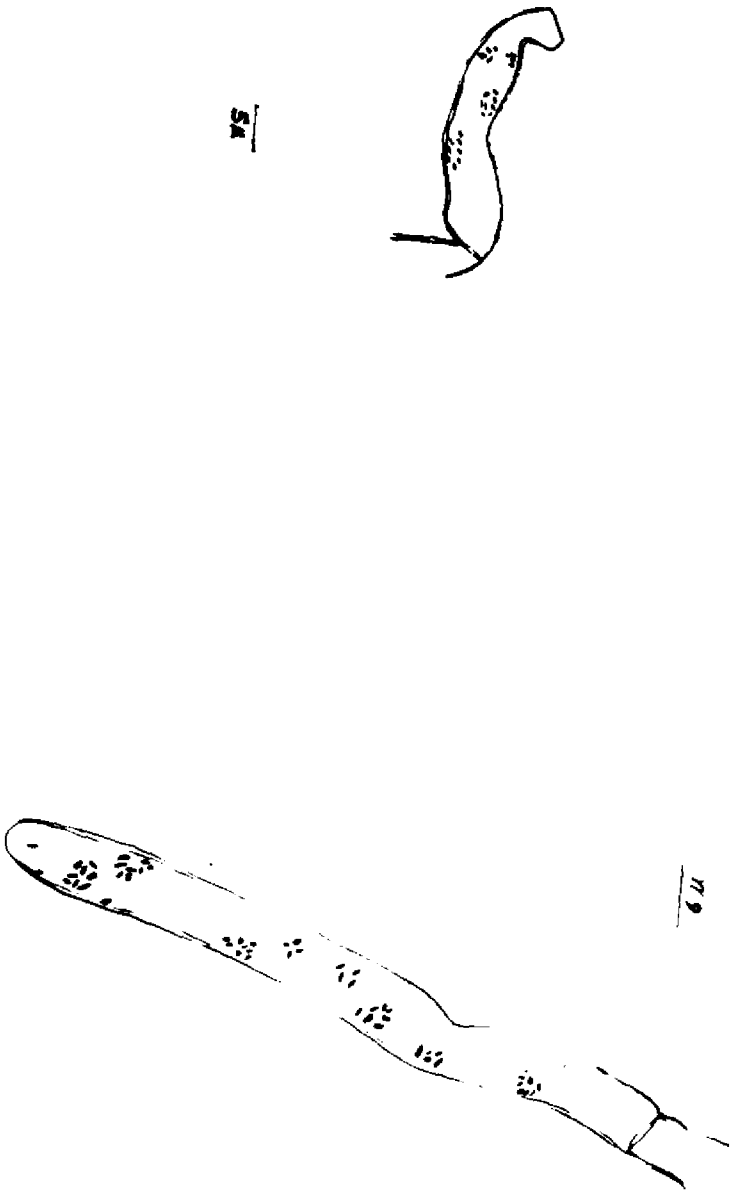

Figure 16. Camera lucida drawings showing nuclei in hyphal tip cells of somatic hyphae of R. solani showing metaphase and multinucleate condition (above and below).

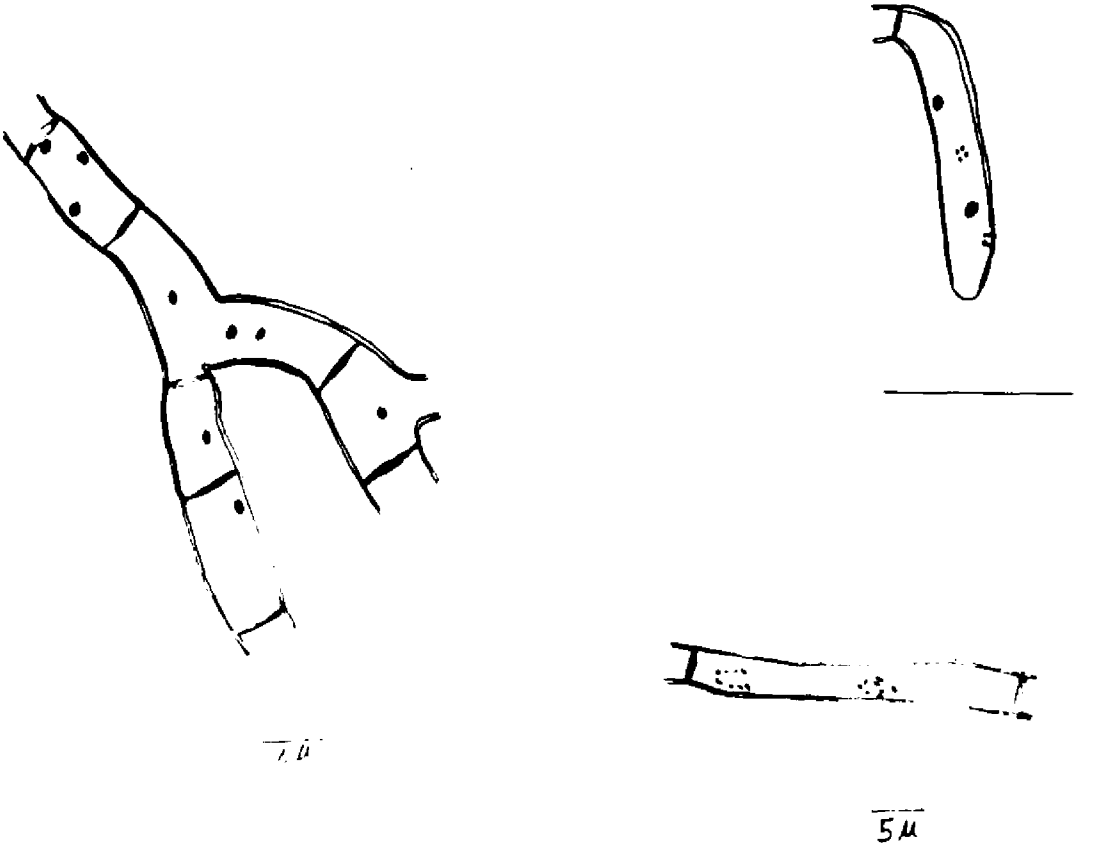

Figure 17. Camera lucida drawings showing nuclei in somatic hyphae of R. solani. Left: resting nuclei in cells of branched hypha. Right: top showing nuclei in resting stage and metaphase; bottom showing nuclei in anaphase.

of the colony. This may have contributed to difficulties of demonstrating nuclear division in somatic hyphae in the past (41, 55). This handicap was overcome in the present investigation with the use of very young cultures, which consisted almost entirely of young, actively growing hyphae.

It was observed, however, that even in mature cells of young hyphae nuclear material degenerated into small particles and was found to be within the size limit of chromosomes. For these studies, therefore, only nuclear division of somatic nuclei in young, hyphal tip cells was used.

The sequence of events in division of somatic nuclei corresponded to that of classical mitosis in higher plants and animals. Flentje (28) reported that all nuclei in a single cell of R. solani divided simultaneously. In the course of this study, no simultaneous division was observed. It was found, however, that all nuclei in a single cell were in one stage or another of mitosis but not all in the same stage. At the beginning of mitosis, nuclei increased in size and chromatic material appeared cup-like or crescent-shaped at the periphery of the nucleolus (Fig. 18, 19). This stage was interpreted as prophase of conventional mitosis. The early stages of prophase were not observed frequently.

The nucleolus soon lost its identity and disappeared. Chromosomes became highly contrasted and could be distinguished at the end of prophase. During metaphase, chromosomes became organized in a typical metaphase plate (Fig. 14, 15, 16, 17, 19, 20, 21). There appeared to be

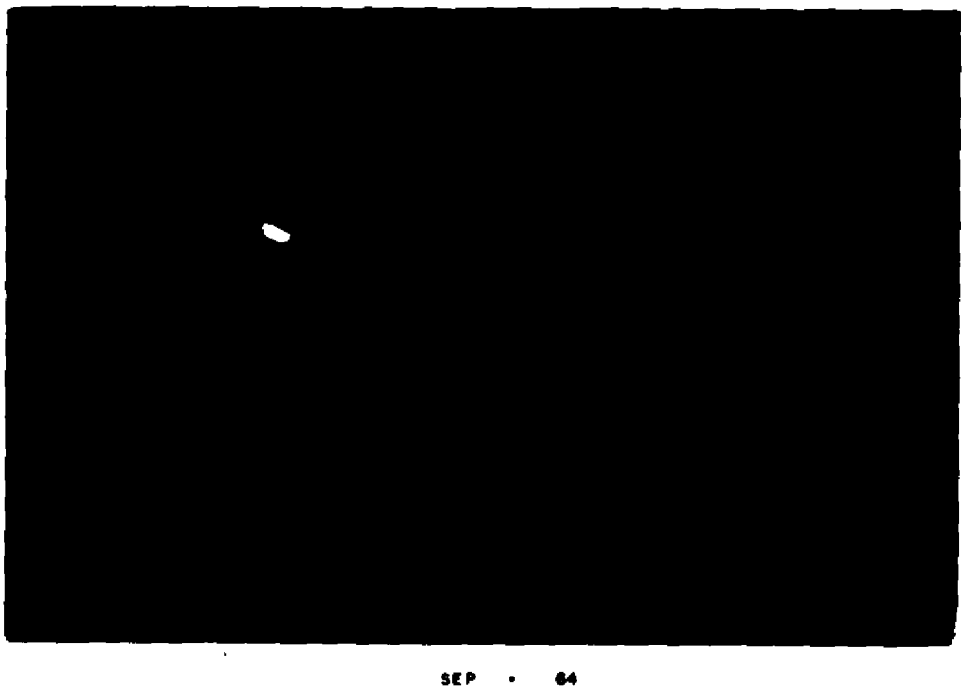

Figure 18. Nuclei in somatic hyphae of R. solani in late prophase. Note chromatic material in crescent shape (see arrow). (X 1200).

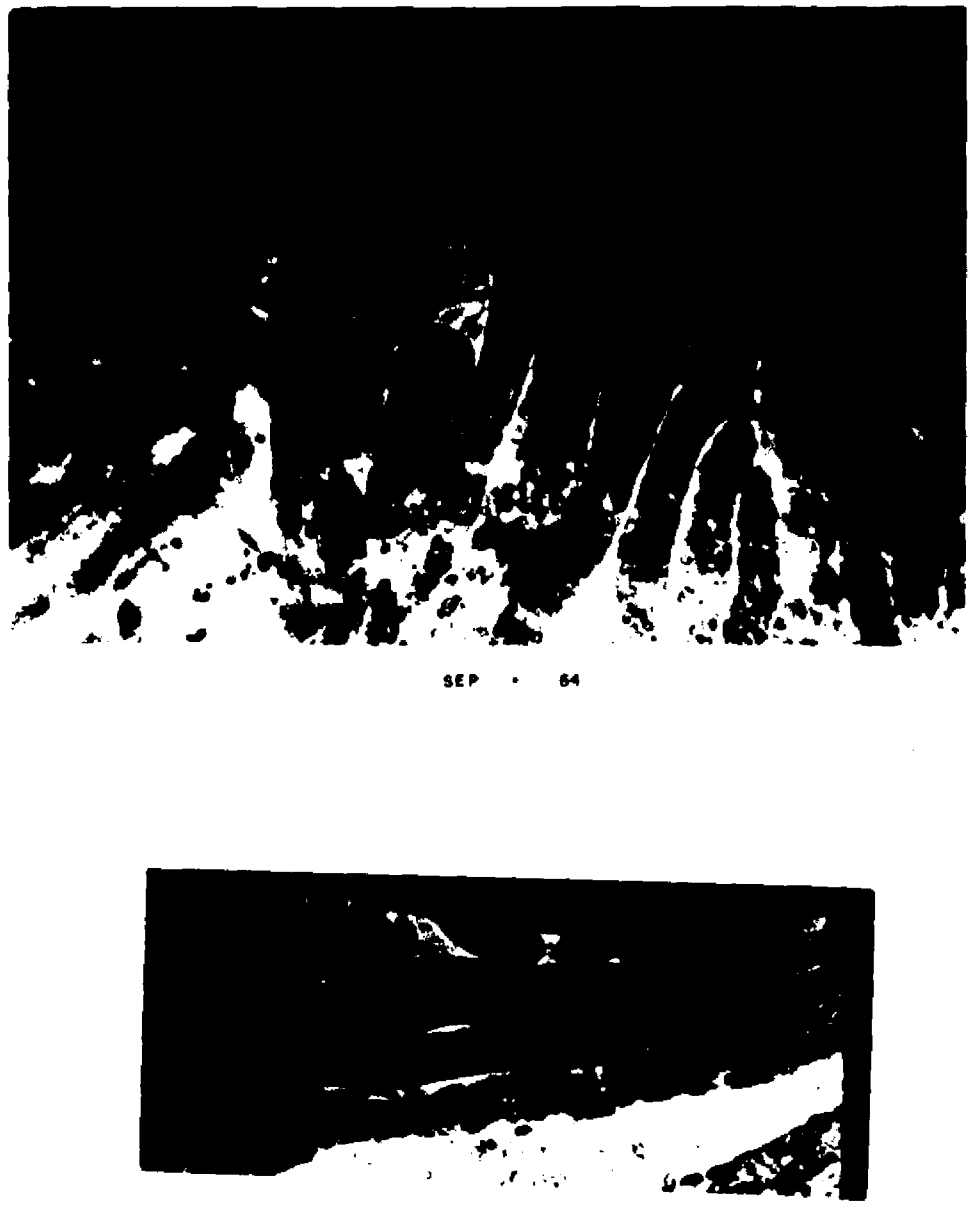

Figure 19. Nuclei in somatic hyphae of R. solani in various stages of mitosis. Upper: nucleus in early prophase (see arrow). Lower: nuclei in metaphase and anaphase (see arrows). (X 1200).

Figure 20. Nuclei in somatic hyphae of R. solani in various stages of mitosis. Upper: nuclei in metaphase (see arrow) and early anaphase (see arrow). Lower: nucleus in mid-anaphase (see arrow). (X 1200).

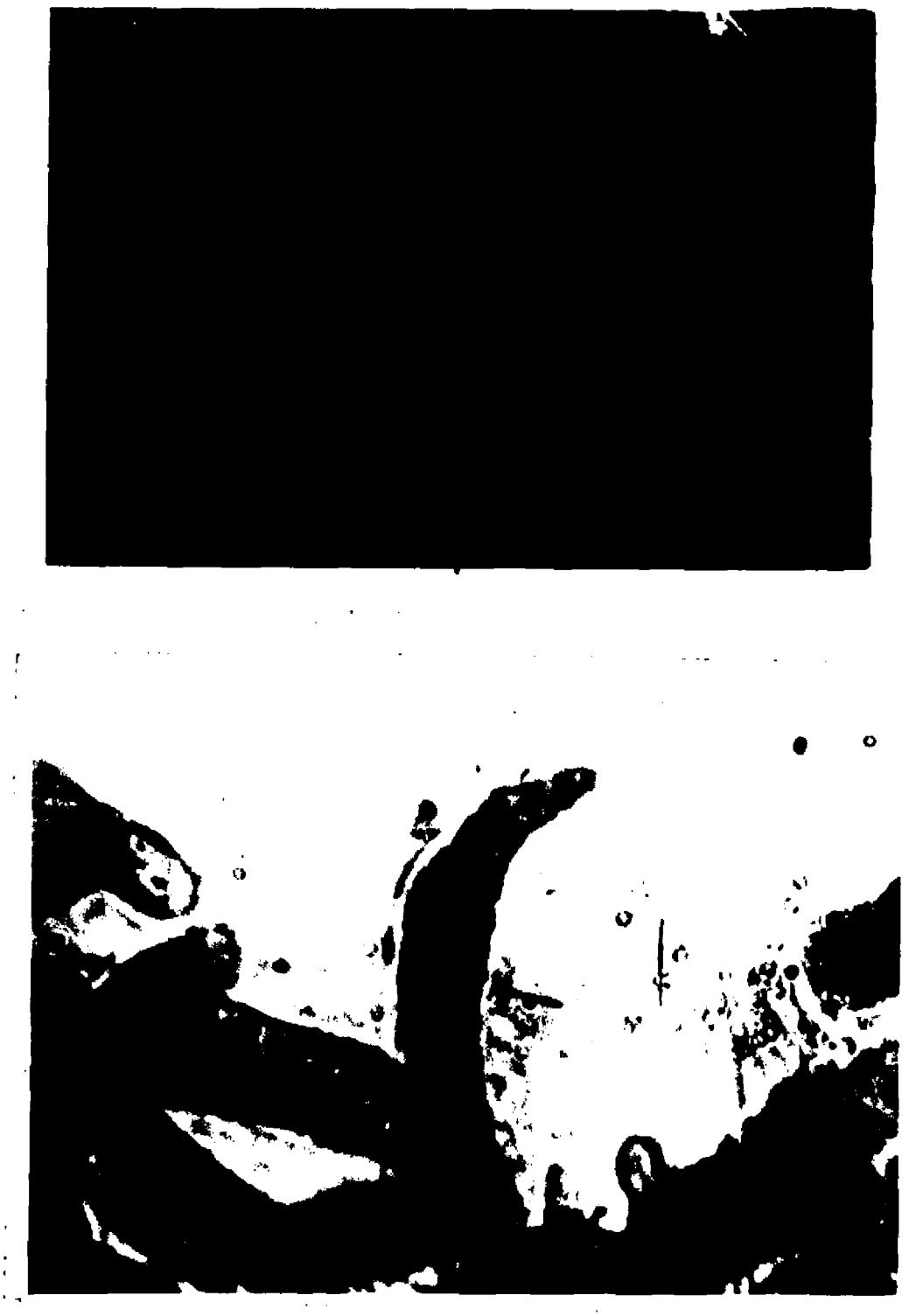

Figure 21. Hyphal tip cell of somatic hypha of R. solani showing metaphase of mitosis. Upper: showing 4 (X 1200) chromosomes (see arrow). Lower: enlargement of upper figure showing chromosomes (see arrow) (X 2000).

4 distinct chromosomes at this stage of mitosis. Chromosome counting was not easy. Chromosomes were in different focal levels, therefore, it was very difficult sometimes to show them in the same plane. Hundreds of chromosome counts were made of nuclei in metaphase and anaphase. The true spindle fibers were not observed, however, many configurations were seen which suggested the presence of a spindle and the separation of the daughter chromosomes to spindle poles (Fig. 22). Anaphase figures appeared showing the chromosomes arranged in a ring-like shape at the polar view (Fig. 15, 16, 17, 19, 20). Telophase nuclei, also, were observed (Fig. 22).

One of the basic characteristics of this fungus was the high frequency of anastomoses between hyphae. This was one of the major factors involved in explaining the variability of this pathogen. It has been suggested that this brings about the intermingling and association of genetically different nuclei. This phenomenon, also, was observed (Fig. 23).

Living nuclei were observed under the phase contrast microscope and were almost always spherical. The nucleus consisted of an optically dense central body, hereafter called the nucleolus, surrounded by an optically less dense area which will be referred to as the halo (Fig. 24, 25). The nucleoli of young nuclei were spherical and occupied a more or less central place within the halo. A true nuclear membrane was observed surrounding these nuclei (Fig. 24, 25). It was observed that cytoplasm consistently flowed toward the tips of growing hyphae. Cytoplasm was found evacuated from older cells in mycelium and passing toward the

Figure 22. Nuclei in somatic hyphae of R. solani in telaphase stage of mitosis (see arrow). Note what appears to be the remains of spindle. (see arrow). (X 1200).

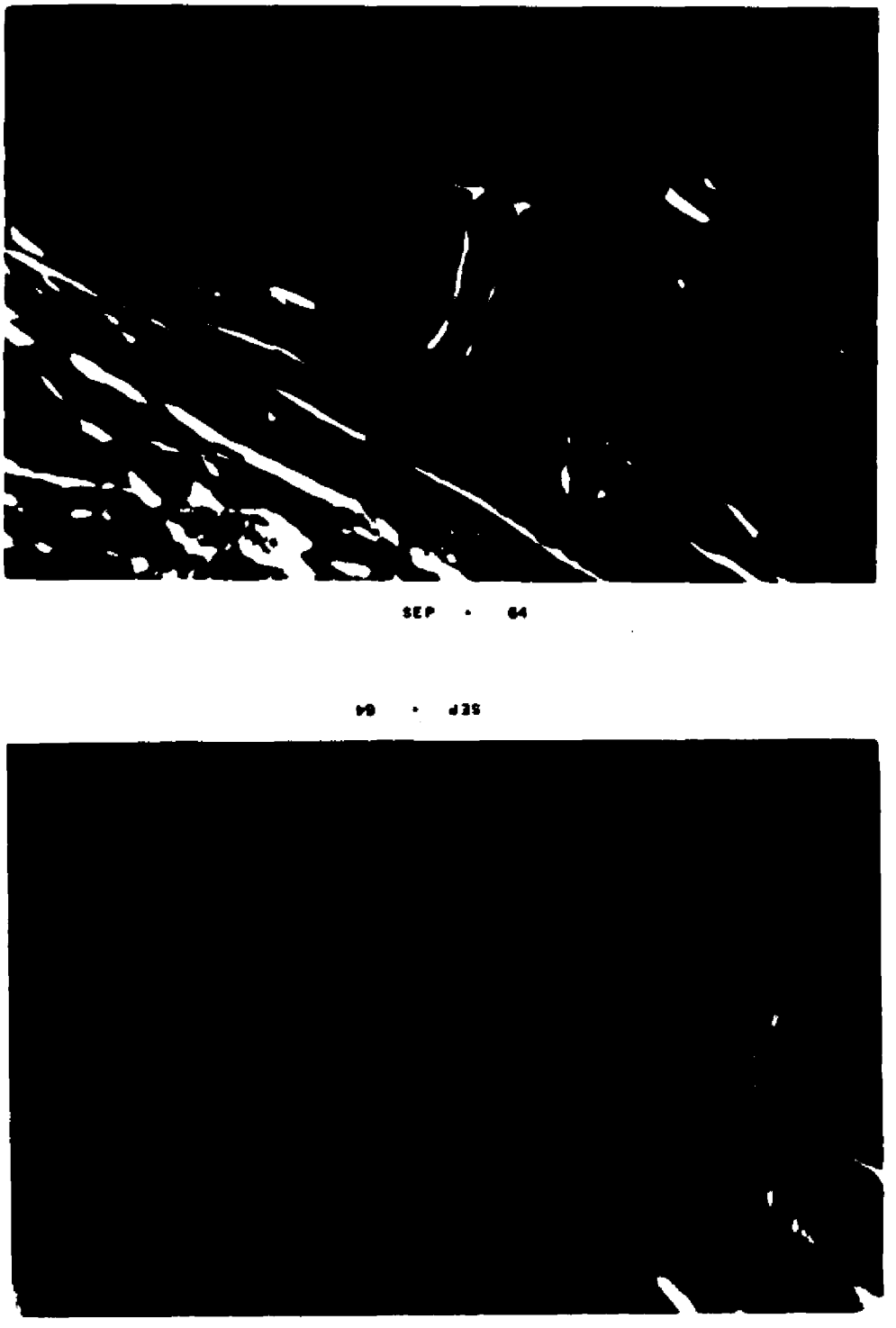

Figure 23. Anastomosis between somatic hyphae of R. solani. Upper: from phase contrast studies of living material. Lower: stained preparation. (X 1200).

Figure 24. Resting nuclei in somatic hyphae of R. solani under phase contrast. Upper and lower: showing halo of chromatic material around nucleoli (see arrows). (X 1200).

Figure 25. Nuclei in somatic hyphae of living R. solani from phase microscope studies. Upper: nucleus in mitotic division (see arrow). Lower: showing multinucleate condition of mycelium cells. (X 1200).

growing hyphal tip. Nuclei of older cells were not carried away in the stream of the cytoplasm, but remained anchored to a fixed layer of cytoplasm that was pressed against the cell wall.

No nuclear migration was observed from cell to cell as claimed by Sanford (69). It was observed, however, that nuclei rotate and move in the vicinity of its position.

Living mycelium was multinucleate and filled with granular and filamentous mitochondria (Fig. 24, 25). It has been difficult to follow the sequence of nuclear division under the phase contrast microscopy. A nucleus was found and interpreted as a dividing nucleus (Fig. 24). It could be differentiated from a resting nucleus by having a larger diameter, the lack of a nucleolus, and the irregular outline of the nuclear boundary.

The ultrastructure of the cell wall consisted of an outer layer of electron transparent material, which has a somewhat filamentous appearance in most sections (Fig. 26). It is composed of several lamellae. The average thickness of this layer was 0.068 u. Beneath this was a layer of electron dense material, which also subdivided into lamellae. The average thickness of this inner layer was 0.12 u.

Plasma lemma or ectoplast bound the endoplasm and were situated just within the cell wall (Fig. 26).

Mitochondria were numerous and clearly defined. In transverse sections they were more or less circular or broadly elliptical, but in longitudinal sections of young hyphae many were elongated (Fig. 27). Mitochondria were surrounded by a double membrane, the inner layer

Figure 26. Electron micrograph of somatic hyphal cell walls of R. solani showing ectoplast electron-dense and electron-transparent layers with lamellae. (X 65000).

Figure 27. Electron micrograph of somatic hyphal cell of R. solani showing ultrastructure of mitochondria. Note cristae. (X 60,000).

projected into the interior forming more or less tubular, irregularly-shaped cristae (Fig. 27). A large number of mitochondria were concentrated in the hyphal tip cells. The average size of mitochondria was 0.6 x 0.26 u.

Nuclei were relatively large, globose bodies with an average diameter of 1.6 to 1.7 u. They showed minor irregularities in the outer lining. The internal mass of nuclei showed no clearly defined structure. Nuclei were surrounded by a clearly defined, double membrane interrupted by nuclear pores (Fig. 28, 29). The nuclear substance was in direct contact with the cytoplasm through these nuclear pores. This was considered the first report on the structure of the nuclear membrane in R. solani. Vacuoles were not present in young hyphae.

Oil drops and food vacuoles were found for the first time in somatic hyphae of R. solani. They appeared as numerous, homogenous, globose bodies. They occurred as clear, white patches of regular outline (Fig. 30). There were more oil vacuoles in hyphal tip cells than in older cells.

The structure of septal pore apparatus appeared typical of what has been reported by Bracker and Butler (10, 11) (Fig. 31). The septum of R. solani hyphae is a complex structure. Near the center of the septum, surrounding the septal pore, was an annular swelling, with a diameter of over 2 u. The swelling grew in a radial direction and did not close the septal pore. When the mycelium grows old the septal pore becomes occluded with electron dense material and protoplasmic continuity between cells is lost. The ectoplast forms the boundaries of the septal swelling.

Figure 28. Electron micrograph of nuclei in somatic hyphal cells of R. solani. Note nuclear envelope with double membrane and nuclear pores. (X 45,000).

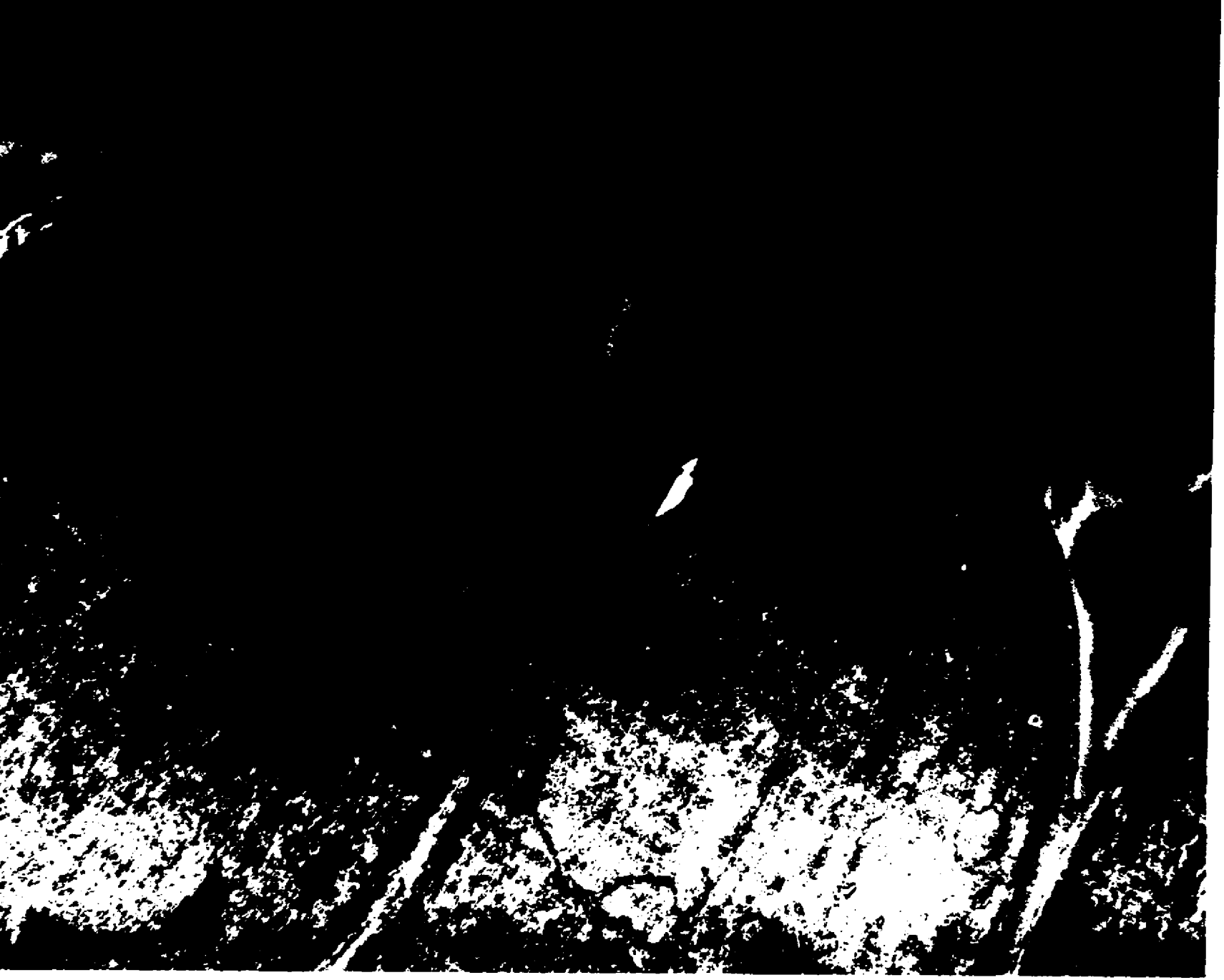

Figure 29. Electron micrograph of nucleus in a somatic cell of R. solani showing ultrastructure of the double membrane of nuclear envelope interrupted by nuclear pores. (X 65,000).

Figure 30. Electron micrograph of somatic hyphal cell of R. solani showing structure of electron-transparent oil vacuoles. delimited by a definite membrane. (X 20,000).

Figure 31. Electron micrograph of somatic hyphal tip cells of R. solani showing septal pore apparatus; concentration of mitochondria in tip cells; oil vacuoles, and nuclei. (X 15,000).

The structure of the septal pore apparatus is one of the most important taxonomic characters for the identification of R. solani (52).

From this observation using stained, living preparations, and ultra-structure studies it was concluded that the somatic nuclei of R. solani have some structural constituents as those of higher plants and they divide by mitosis as in higher plants.

DISCUSSION

Part II

In the course of sexual reproduction the nuclei of many fungi give rise to chromosomes of more or less ordinary appearance and behavior. The literature concerned with this phase of fungal life is extensive and records the occurrence of meiotic and mitotic nuclear division in such cells. By contrast there are few adequately illustrated descriptions of the structure and division of somatic nuclei.

There has not been agreement among investigators on the occurrence of mitotic division in somatic nuclei. Some felt that nuclear division was not similar to classical mitosis, but was characterized by elongation of the nucleus, constriction and separation of daughter nuclei without the appearance of individual chromosomes or the organization of mitotic figures. It seems premature to deny occurrence of somatic mitosis regardless of the misleading conclusions of those investigators who fail to demonstrate typical mitotic figures in the somatic fungal nuclei.

Cytological studies of fungi are attended by appreciable technical difficulties. The main difficulty in studying fungus nuclei is their small size. Other problems are difficulty of spreading the chromosomes within the nucleus, the small differences in technique may greatly affect the appearance of stained nuclei; and a procedure which gives good results with one fungus may not be satisfactory for others. Optimum staining is

necessary. Over-stained preparations always lead to misleading interpretation for neither individual chromosomes nor stages of division can be distinguished. The combination of these difficulties may account for many of the failures in attempts to demonstrate mitotic division in somatic nuclei of fungi.

It does not appear likely that there would be 2 modes of nuclear division, meiotic and mitotic division in the sexual cells and another mode of division in the somatic cells. Particularly strong evidence of the presence of chromosomes in somatic nuclei and of the occurrence of nuclear division by mitosis was confirmed in studies of mitotic recombination in fungi by Pontecorvo and Kaffer (56).

Robinow (61) contended that a classification of living things into higher organisms and protists could be correlated using differences in nuclear division. He considered mitotic and meiotic division as criteria for classifying higher organisms, and other methods of nuclear division for the protists. This latter group embraced fungi along with the bacteria, slime molds, protozoa, and algae. Robinow and Bakerspigel (1, 2, 3, 4, 5, 6, 7, 61, 62, 63, 64, 65) attempted to provide evidence in support of this classification of the fungi.

In amitosis no separate stages of division can be observed, the entire nucleus divides into 2 portions. Amitosis provides no evidence of equal distribution of chromosomes to daughter nuclei and therefore no guarantee of the continuity of genetic material in successive nuclear generations.

Some illustrations of Bakerspigels (1, 4, 5), who published extensively in support of direct division in somatic nuclei of fungi, showed occurrence of complexes of chromosome-like elements in hyphae of Blastomyces dermititidis, N. crassa, and Gelasinospora tetrasperma. He reported that individual chromosomes were not always noted and at the same time recorded the chromosome number.

No clear indication was given as to how these observed chromosomes fit into the scheme of division that was not mitotic. Somers, Wagner, and Hsu (79) believed the difference between their observations and those recorded by Bakerspigel, in division of somatic nuclei in N. crassa, was only in interpretation. They mentioned that division figures in Bakerspigel's photographs were similar to their observations. What he considered as complexes of chromosomal filaments were somewhat comparable to metaphase configurations in which chromosomes had not been spread sufficiently to show their individual morphology.

It was believed by Ward (90, 91) that the failure of Bakerspigel to demonstrate somatic mitosis in his studies was due to the technique used, which, in most cases, showed overstained preparations and condensed chromatic material. The writer believes that another factor was Bakerspigel's position concerned with the scheme of classification of microorganisms and his efforts to support this classification.

There is no doubt that the technique used for studying stained preparations reported here contributed to the success in studying somatic nuclear division in R. solani.

The observations described in this manuscript provided conclusive evidence that somatic nuclei of R. solani divide by mitosis in essentially the same manner as in higher plants and animals. The most elementary criteria of mitosis were: demonstrable chromosomes; their alignment on a metaphase plate; and separation of daughter chromatids into daughter nuclei. Further studies are needed to detect the spindle apparatus and its formation.

It was observed in this study that the somatic cells were multinucleate and the number of nuclei was greatly increased in hyphal tip cells sometimes reaching to 15. This observation agrees with all studies of somatic cells made in the past by Fukano (29), Hawn and Vanterpool (33), Muller (48), and Sanford (69). Although there was no indication in any of these studies of the mechanism by which it was achieved, it was observed by the writer that the number of nuclei per cell decreased greatly in cells behind the hyphal tip. This agreed with observations reported by Muller (48), Fukano (29), and Sanford (69).

In our study no nuclear migration was observed which was in agreement with Flentje, et al. (28). Saksena (67, 68), Sanford (69), and Fukano (29), however, observed nuclear migration in R. solani hyphae. They interpreted the change in nuclear number per cell to nuclear migration. Flentje, et al. (28), however, believed that it was due to either the development of secondary septa in older cells without nuclear division or to the variability in the behavior of nuclei during division.

Stained and living preparations of old cells showed that the nuclei were restricted to the hyphal cell wall and few in number. It was believed that in old cells, after the cytoplasm was evacuated by protoplasmic streaming, nuclei remained adjacent to the cell wall and some of these nuclei began to degenerate.

The occurrence of mitotic nuclear division in the somatic nuclei of R. solani was observed. The nuclei within one cell were observed dividing in close stages of mitosis but not simultaneously. This study does not agree with Flentje, et al. (28), who recorded the occurrence of simultaneous nuclear division in nuclei of this fungus. They mentioned that the nuclei split into chromosomes at the time of division and that they could not find any support for the suggestion by Saksena (67, 68). Saksena (68) held that these nuclei divide by elongation, constriction, and separation as described by Robinow (62, 63) and Bakerspigel (1, 2, 3, 4, 5, 6, 7, 8). Flentje, et al. (28) suggested that the interpretations made by Saksena (67, 68) were due to over-staining. They did not, however, mention or report about the kind of nuclear division that took place in such cells.

The (n) chromosome number appeared to be 4, which did not agree with Hawn and Vanterpool (33), who reported 6 chromosomes. Olive (51), reported in his review article that the haploid chromosome number most frequently found in the Hymenomycetes was 4. The demonstration in this study, therefore, of a haploid chromosome number of 4 is likely to be reasonable.

The nuclei were observed, also, under a phase contrast microscope, but it was difficult to follow the sequence of events during division. This difficulty can be attributed to (2): 1) Continuous observation of a single nucleus was usually obscured; 2) the large number of nuclei packed within a single hyphal cell; and 3) the use of various focal levels created difficulty in continuous observation of a single nucleus during division and post division.

The ultrastructure of R. solani hyphae has been greatly furthered, using the electron microscope during this study. The structure of the cell wall was revealed and described. The ultrastructure of the nuclear membrane was demonstrated and described for the first time and found to be comparable to that of higher plants. The presence of oil vacuoles was demonstrated in R. solani hyphae for the first time. Mitochondria and other organells in the cell were observed.

The septal pore apparatus was demonstrated and was comparable to the description by Bracker and Butler (10, 11).

From this study it was concluded that the somatic nuclei of R. solani divide by mitosis comparable to that observed in higher plants. The structure of the somatic nuclei in R. solani was found to be similar to that found in higher plants.

This study confirmed the theory of the occurrence of mitotic division in somatic nuclei of fungi. Such study is of great significance for those investigators who are searching for the origin and evolution of fungi.

SUMMARY

Part I

1. Some 52 cotton varieties, lines, strains, and species were tested for their reaction to 3 isolates of R. solani using controlled temperatures, greenhouse and field conditions.
2. The 14 Upland cotton varieties of G. hirsutum showed great variation in their reaction to R. solani.
3. Reaction of the varieties to any given isolate varied with environmental conditions, especially temperature. Deltapine smooth leaf was considered resistant to isolate T under greenhouse conditions, while it appeared susceptible under controlled temperatures of 20-24°C.
4. Under greenhouse conditions Upland varieties, as a group, were considered more susceptible to R. solani than the Sea Island group, G. barbadense.
5. Resistance of cotton varieties to R. solani isolates behave as any other biological phenomenon, that is, showing a normal curve.
6. Irradiation of cotton seed appeared to have some effect on resistance of these lines to R. solani.
7. Irradiation of 500 roentgens appeared to increase tolerance to infection of these lines compared to nonirradiated lines.

8. These results showed promise for further evaluation of these lines in attempting to make use of irradiation as a means of developing some lines resistant to R. solani.

9. These tests for evaluating cotton varieties either under controlled temperature or under greenhouse conditions were considered severe conditions in comparison to natural conditions.

10. A larger number of varieties, lines, and species from all over the world should be tested for their resistance to a larger number of R. solani isolates.

11. The reaction of cotton varieties, lines and strains, to the seedling disease complex under natural conditions was determined in both Red River Valley and Northeast Louisiana Branch Experiment Stations.

12. Variation from resistant to susceptible of these varieties was observed under field conditions.

13. The reaction of these varieties differed according to the location and season.

14. Differences in reaction of these cotton varieties were used to establish physiologic specialization of R. solani isolates.

15. The glandless cotton lines, and Yugoslavian strains showed promise in their resistance level to the seedling disease complex under field conditions.

16. Continuous work is suggested to establish physiologic specialization of the pathogen on cotton and to determine the predominance of parasitic races in different locations.

17. This study was considered as a step forward in establishing physiologic specialization among isolates of R. solani from cotton. This was considered of great importance from the practical, as well as, the scientific standpoint.

Part II

1. The cytology of isolate T of R. solani was studied using the light, phase, and electron microscopy.
2. In differential staining using a modified aceto-carmine-squash technique, the diameter of hyphae was found to be about 7.0 u. Cells were multinucleate, with the number of nuclei greatly increased in hyphal tip cells, the number sometimes reaching 15.
3. In old cells the number of nuclei was reduced and nuclei appeared to be compressed to the cell wall. Nuclear degeneration occurred in such cells.
4. Diameter of nuclei in the resting stage ranged from 1.6 to 1.7 u. Nuclei appeared as spherical, dark-stained bodies with a definite border suggesting the presence of a nuclear membrane.
5. Nuclear division was studied in hyphal tip cells where young, actively-dividing nuclei existed.
6. It was observed that somatic nuclear division was mitotic and comparable to that found in higher plants.
7. Spindle apparatus was not observed.

8. The (n) chromosome number in somatic nuclei appeared to be 4 as observed in metaphase and anaphase figures.

9. Living material, under the phase contrast microscope, showed that resting nuclei contained a nucleolus which appeared as a spherical, optically dense body surrounded by a halo of chromatic material. The nuclear membrane was observed.

10. Phase-contrast microscopy confirmed observations made of stained preparations.

11. Nuclear division was not observed in living preparations. A nucleus, which appeared to be dividing, however, was noticed. It was characterized by its larger size and irregularities of its boundary.

12. Nuclear migration was not observed, cytoplasmic streaming from cell to cell in direction of the hyphal tip was noticed.

13. The ultrastructure of R. solani hyphae was revealed using the electron microscope.

14. The cell wall was composed of 2 layers, an outer layer made of electron-transparent material and an inner layer made up of an electron-dense material. Each layer was composed of lamellae.

15. The ultrastructure of the nuclear envelope was described for the first time. It was found to be composed of two double layers interrupted by nuclear pores. It was comparable to that in higher plants.

16. The demonstration of oil vacuoles in hyphal cells of R. solani was shown for the first time. They appeared as circular, white patches surrounded by a definite membrane.

17. The ultrastructure of mitochondria and other organells in the cell was shown.

18. The ultrastructure of the septal pore appratus was shown and appeared to be typical of the description by Bracker and Butler (10, 11).

19. This study confirmed and added to the literature the occurrence of mitotic nuclear division in the somatic nuclei of R. solani, and showed the ultrastructure of some cell constituents for the first time. It is of considerable importance for those who attempt to search for the origin and evolution of fungi.

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VITA

Mohamed-Nagy Mahmoud Shatla was born in Shebin-El Kom, Egypt U.A.R. on December 12, 1939. In 1946 he entered the primary school, where he was graduated in 1950. In the same year he enrolled at Shebin El Kom secondary school from which he was graduated in 1955. During the same year he enrolled at Cairo University College of Agriculture where he received the B.S. degree in 1959. He served as instructor in the Horticulture Department in the Higher Institute of Agriculture at Shebin El Kom from December 1959 to July-1961. At that time he was chosen by the U.A.R. Government to complete his studies toward the Ph.D. degree in Plant Pathology in the U.S.A. He spent the summer of 1961 at Boston University for an orientation course. In the fall of 1961 he enrolled at Louisiana State University in the Department of Botany and Plant Pathology. He received the M.S. degree in January 1963. He was elected to Phi Kappa Phi (Fall, 1963), Gamma Sigma Delta (Spring, 1964), and to Sigma Xi (Spring, 1964). He is a candidate for the degree of Doctor of Philosophy in Plant Pathology in January 1965.

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EXAMINATION AND THESIS REPORT

Candidate: Mohamed-Nagy Mahmoud Shatla

Major Field: Plant Pathology

Title of Thesis: Physiologic Specialization and Cytology of Rhizoctonia solani from Cotton

Approved:

James B. Kinelair
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

M. T. Henderson

J. C. Forbes

B. Lowry

M N Escalopley

St John P. Clinton

Date of Examination:

October 22, 1964